





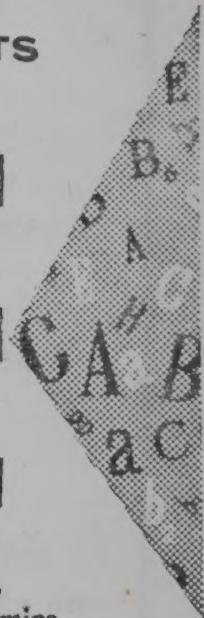
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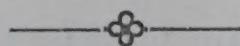
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# ENZYMES

I. S. BHATIA

Scientific Department, Indian Tea Association Tocklai  
Experimental Station, Cinnamara

Investigations covered in this year's review do not differ appreciably from those of last year. This year a large number of enzyme systems has been investigated including oxidative and proteolytic enzymes, esterases, lipases, phosphatases and also a few synthesizing enzyme systems, etc.

One aspect of enzyme field which has not received due share of attention by the Indian workers is the mechanisms of enzyme action and the relationship between structure and enzymic action. This is just as much true of this year as of the previous years.

## OXIDATIVE ENZYMES

### Dehydrogenases

The dehydrogenase activities of susceptible and sulfanilamide-resistant strains of *B. subtilis* grown in peptone or semi-synthetic media were studied by Joshi and Sreenivasan<sup>1</sup> using glucose, glycerol, calcium lactate, sodium pyruvate and ethyl alcohol as substrates. The resistant strain showed a marked decrease in dehydrogenase activities with all these substrates. The behaviour of the cell-free extracts of susceptible and resistant strains was comparable to that of the corresponding intact cells.

Rindani<sup>2</sup> showed that the increased succinic dehydrogenase activity in the liver homogenates of castrated male rats was counteracted by administration of testosterone propionate. It was also shown that the enzymic activity was not related directly to the weight of the liver.

Kothare and DeSouza<sup>3</sup> demonstrated cytochemically the presence of succinic dehydrogenase, using p-nitrophenyl substituted ditetrazole, in normal, immature and abnormal spermatozoa from semen of normal men. The enzyme was present in the head and mid-piece and absent in the neck and tail.

## Oxidases

Rao *et al.*<sup>4</sup> have reported on the *in vitro* action of "Makaradhwaja" (red mercuric sulphide) employed in Ayurvedic system of medicine as a tonic, on succinic oxidase, succinic dehydrogenase and cytochrome oxidase of rat liver homogenate. It has been shown that the drug inhibits succinic oxidase and dehydrogenase but has no effect on cytochrome oxidase.

## NON-OXIDATIVE ENZYMES

### Lipases

Lakshminarayanan<sup>5</sup> studied lipase formation by *Fusarium vasinfectum* Alk. and also the factors controlling the lipolytic activity and the optimal conditions for maximal activity.

Ramakrishnan<sup>6</sup> analysed different lipase preparations obtained from *A. niger* for their amino acid composition. The ammonium sulphate fraction which has got the higher lipase activity, consists of the following amino acids: cystine, arginine, histidine, glycine, glutamic acid, threonine, alanine, methionine, valine, phenyl alanine, leucine, isoleucine and tyrosine.

Borkar and Ghosh<sup>7</sup> investigated lipase activity in mycelia and culture fluid of *P. chrysogenum* during commercial penicillin fermentation. It was found that the enzyme is progressively secreted into the culture fluid. The implications of the results are discussed with respect to the commercial practice of using oils as antifoam agents in penicillin fermentation. Mycelial lipase activity was found to be stimulated by cysteine and inhibited by iodoacetate and para chloromercuribenzoate whereby it is concluded that the enzyme is—SH sensitive.

George and Scaria<sup>8</sup> made a chemical study of the distribution of lipase in pectoralis major muscle of the pigeon. Their results indicated that lipase occurs mostly in the fat-loaded narrow fibres and very little, if any, in the glycogen loaded broad fibres. Similar conclusions were reached by George *et al.*<sup>9</sup> in their investigations on lipase activity in the pectoralis major muscle of the bat.

In another publication<sup>10</sup> the histochemistry of muscle lipase is discussed. A lipase was found in the heart muscle of pigeon and the narrow fibres of pectoralis major of dove. Muscle lipase was

destroyed by acetone fixing and to prevent enzyme loss, tissues from test animals were frozen over ice in a freezer and sections cut therefrom with a cold sharp razor and mounted on albuminized slides for investigations.

### Esterases

Krishnamurthy *et al.*<sup>11</sup> studied the intracellular distribution of vitamin A esterase and cholesterol esterase in chicken liver. Homogenates of chicken liver prepared in 0.25 M sucrose were fractionated by means of differential centrifugation into nuclear, mitochondrial, microsomal and supernatant fractions. Whereas in the rat liver, vitamin A esterase, cholesterol esterase and esterase activities were located solely in the microsomal fraction, in the chicken liver, they were distributed between nuclear and microsomal fractions.

### Phosphatases

Bhattacharjee *et al.*<sup>12</sup> have reported on the alkaline phosphatase activity in *Trichophyton rubrum*, which was shown by them to vary with the age of the fungus and nature of the culture media.

Kar and Das<sup>13</sup> studied the effects of aldosterone monoacetate and deoxycorticosterone acetate on alkaline phosphatase activity in the liver of rats. Qualitatively the two hormones have similar effect on the enzyme; but they differ with respect to degree; deoxycorticosterone acetate being three times more effective, as an inhibitor, than aldosterone monoacetate.

Chanda<sup>14</sup> has reported on the effect of oral administration of thyroxine to cows and buffaloes on the phosphatase level in the blood and milk of the animals. Phosphatase activity decreased in the milk and increased in the blood serum. The correlation of phosphatase activity to tocopherol and ascorbic acid in milk and blood are discussed.

George *et al.*<sup>15</sup> have reported on alkaline phosphatase activity in the pigeon breast muscle. These authors who distinguish between broad and narrow fibres in transverse sections of pectoralis major, report that the alkaline phosphatase reaction

1\*

is intense in the narrow fibres and found only faintly in the sarcolemma of the broad fibres.

### **Urease**

Nath and Mukherjee<sup>16</sup> have reported on the preparation of a urease from *Cajanus indicus* which was found to be satisfactory for blood urea determinations.

### **Catalase**

Seshachar *et al.*<sup>17</sup> have reported on the isolation and properties of catalase from a *Blepharisma* species. The enzyme is best extracted by homogenizing the animals in distilled water. The optimum pH and temperature for the activity of the enzyme are 7.0 and 30°C respectively. The enzyme is inactivated at temperatures above 50°C. Dialysis of the enzyme extract decreases the activity of the enzyme which is completely inhibited at a concentration of 3 micromoles of potassium cyanide and 10 micromoles of sodium azide.

Ghatak *et al.*<sup>18</sup> showed that the five antigenic strains of *S. typhosa* examined by them possess catalase activity, with a pH optimum of 7.5. Young cells were more active than older ones. The inhibitory effect of antibiotics is also reported.

## **ENZYMES INVOLVED IN CARBOHYDRATE METABOLISM**

### **Starch and Sucrose Synthesising Enzymes**

Ramakrishnan<sup>19</sup> studied the synthesis of sucrose and starch in the sugar-cane. It was found that the concentration of the sucrose synthesizing enzyme decreases with maturity of the plant. Starch synthesizing enzyme could be located only in the top node of the matured sugar-cane, which happens to be the only site where starch could be detected.

An investigation by Chatterjee *et al.*<sup>20</sup> into the mechanism of utilization of carbohydrates by *Leishmania donovani*, the protozoan causing visceral leishmaniasis, revealed the presence of strong hexokinase activity in the cell free extract of this organism. By a study of the inhibition and activation of these enzymes the authors concluded that hexokinase of *L. donovani* is not markedly dependent on functional—SH groups for its activity.

The utilization and synthesis of oligosaccharides by two species of *Pestalotia* has been reported upon by Tandon and Bilgrami.<sup>21</sup> The two species used were *P. banksiana* Cavara (isolated from diseased leaves of *Grevelia robusta*) and *P. citri* Mundkur and Keshwala (isolated from the diseased leaves of *Citrus grandis*). These were isolated from their respective hosts and grown on a basal medium containing in addition six oligosaccharides. The filtrates were analysed chromatographically and the sugars were identified by their  $R_f$  values. It was shown that raffinose was utilised through a hydrolytic pathway by both the species of *Pestalotia*. Melibiose and fructose were detected by paper partition chromatography. Cellobiose, lactose and melibiose were poorly utilized through a non-hydrolytic pathway. Maltose was the sugar best utilised by the organisms. They converted maltose by transglucosidation into maltotriose with simultaneous liberation of glucose.

### Pectic Enzymes

Lakshminarayanan<sup>22</sup> has reported on some of the factors such as substrate concentration, pH, temp., etc., governing the activity of pectin methyl esterase (PME) from *Fusarium vasinfectum*. It has been reported that the enzyme resembles other mold PME preparations in certain respects and the plant PME in others. The normal healthy tissues of susceptible cotton plant contains a very low level of the enzyme while wilt-infected ones record much higher activity, especially at the site of infection, viz., the root region.

In connection with the work on pectic enzymes of parasitic fungi, Gupta<sup>23</sup> has reported on the secretion of protopectinase enzyme by *Pythium de Baryanum* Hesse using different potato media. Potato decoction and extracted sap of frozen potato tubers form good media for the secretion of the enzyme. Minced potato extract is not a suitable medium for this purpose. The amount of growth of the mycelium had no relation to protopectinase activity.

Kartar Singh *et al.*<sup>24</sup> reported on the presence of a pectin depolymerase in the penicillin fermentation broth and mycelia

which resembles the pectic depolymerase of fungal origin. This enzyme reduces the viscosity of pectic solutions without causing any increase in the reducing groups. The enzyme activity was followed using pectin and pectic acid as substrates and measuring the fall in viscosity. Galacturonic acid could not be detected as the end product of the reaction indicating a low pectinase activity.

## ENZYMES INVOLVED IN PROTEIN METABOLISM

### Proteolytic Enzymes

It has been reported by Bahadur and Sinha<sup>25</sup> that papain proteolysis of casein is activated by vitamin C, alone or with Fe<sup>++</sup> or Mn<sup>++</sup>, in a degree similar to the effect of cyanide.

They studied<sup>26</sup> the relative proteolytic activities of trypsin and papain and found the following order: activated papain > trypsin > non-activated papain. The order of the substrates capable of undergoing hydrolysis was, with trypsin, casein > heated egg-white > meat extract > unheated egg white > ; with activated papain, the order was casein > egg-white > meat extract. These authors<sup>27</sup> separated commercial papain into 7 crystalline fractions varying in solubility in ethanol, in refractive indices, iso-electric points and proteolytic action on casein.

Bose and Krishna<sup>28</sup> have reported on the purification and properties of the protease from the latex of madar plants (*Calotropis gigantea*).

Chatterjee *et al.*<sup>29</sup> studied the mechanism of blood coagulation by proteolytic enzymes. It was shown that heparin in lower concentration extends the clotting time of oxalated rabbit plasma with decalcified trypsin and in larger amount prevents clotting altogether. Similar results were obtained with decalcified papain. Sodium salt of ethylene-diamine-tetraacetate at a concentration of  $0.2 \times 10^{-3}$  inhibits the clotting of trypsin, but above  $.05 \times 10^{-3} M$ , it accelerates the action.

In the succeeding paper<sup>30</sup> the authors report that the clotting activities of trypsin and papain are lost only when heated at 70° and 85°C respectively even though proteolytic activities are destroyed at lower temperatures, i.e., 60° and 75°C respectively.

### Protein phosphokinase

Sundararajan *et al.*<sup>31</sup> prepared a partially purified protein phosphokinase from rabbit mammary gland by fractionation with acetone followed by ammonium sulphate fractionation. Using P,<sup>32</sup> it was shown that the enzyme could phosphorylate proteins and that the phosphorus uptake by the proteins was increased after partial dephosphorylation; complete dephosphorylation, however, appeared to decrease activity.

## ENZYMES INVOLVED IN AMINO ACID AND NITROGEN METABOLISM

### Deaminases

The presence of an enzyme capable of releasing free nicotinic acid and ammonia from nicotinamide in stoichiometric proportions has been demonstrated in the cell-free extracts of *Neurospora crassa* by Sundaram *et al.*<sup>32</sup> By fractionation of *N. crassa* into its sub-cellular components, it has been shown that the nicotinamide deaminating activity is localized in the soluble portion of the cytoplasm. The formation of this enzyme is independent of the presence of nicotinamide or nicotinic acid in the culture medium.

A study on the distribution of the above enzyme in the animal kingdom<sup>33</sup> revealed its absence in the tissues of rat, guinea pig, sheep, hog and cattle, its presence in the liver of the rabbit in small amount and its high concentration in the liver and kidney of the pigeon and the kidney of the chick. Based on these findings the authors conclude that the enzyme is of specific nature distinct from ordinary peptidases or similar enzymes acting non-specifically.

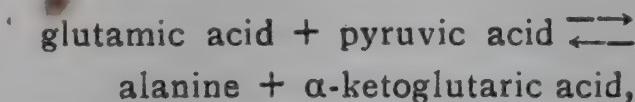
Ghatak *et al.*<sup>34</sup> reported on the deamination of amino acids by an enzyme from some antigenic strains of *Salmonella typhosa*. Using twenty-one amino acids as substrates, it was shown that the ammonia production from aspartic acid and serine was maximum followed by arginine, alanine, cysteine and glutamic acid. L-phenyl alanine, tryptophane and homocysteine showed little or no deamination. The order of activity for the various strains was not uniform and was found to be different for each amino acid,

$Hg^{++}$ ,  $Ni^{++}$ ,  $Cd^{++}$ ,  $Cu^{++}$  and  $Zn^{++}$  possessed inhibitory action whereas  $Mg^{++}$  had a pronounced activating effect.

### Transaminases

Patwardhan<sup>35</sup> prepared an aspartic-glutamic transaminase from fresh green leaves of *Dolichos lablab*. By treatment of the residue after acetone extraction of the leaves with 'Dowex 2', the enzyme was obtained in a high state of purity as shown by paper electrophoresis.

Das and Rao<sup>36</sup> studied the effect of benzyl, isobutyl and allylisothiocyanates on the green gram transaminase catalyzing the following reaction :



Varying degrees of inhibition were observed with all the three isothiocyanates.

### Decarboxylases

Natarajan<sup>37</sup> observed the presence of  $\gamma$ -amino butyric acid in *Fusarium vasinfectum*. A study of the enzyme system revealed the presence of glutamic acid decarboxylase, i.e.,  $\gamma$ -amino butyric acid was formed by the decarboxylation of L-glutamic acid.

Nadkarni and Sreenivasan<sup>38</sup> have reported the presence of serine decarboxylase in the mitochondria of rat liver.

### Enzymic Synthesis of Nicotine Alkaloids

Bose *et al.*<sup>39</sup> reported on the extraction of an enzyme from the leaves, roots and stems of *N. tabacum* and *N. glauca* by acetone precipitation of the cold water extract of the ground tissues. They observed that synthesis of nicotine is accelerated by the addition of amino acids and inorganic nitrates *in vitro* as well as *in vivo*.

## SPECIAL SYSTEMS

### Ascorbic Acid Synthesizing System

Guha<sup>40</sup> reported on the presence of a specific substrate system for the synthesis of ascorbic acid by animal liver. Of the

substrates examined, viz., Na glucuronate, Na galacturonate, Na pyruvate, Na pyruvate + dihydroxy acetone, D-glucuronolactone, etc., only the last was found to be effective. Ascorbic acid formation did not take place in the absence of  $CN^-$ , nor in the absence of air. Rat, mouse, rabbit, cow and goat livers were effective. None was formed from guinea pig, pigeon or chicken liver. Ascorbic acid was detected by indophenol titration. Potent enzyme concentrates were obtained, after sedimentation of nuclear material and mitochondria, by ammonium sulphate fractionation between 30 and 50% saturation. Goat liver extracts retained almost full activity for 3 days at  $5^\circ$  but only 25% after 2 weeks. No co-enzymes or co-factors were necessary for the reaction.

Guha and co-workers<sup>41, 42</sup> have further shown that the enzyme responsible for the synthesis of ascorbic acid resides entirely in the microsomal fractions of the liver tissues of goat and rat, the supernatant fraction having an inhibitory rather than a stimulatory effect on this synthesis. The synthesizing ability of the microsomal fraction was fully retained even after dialysis. Potassium cyanide, without any added co-factor, was shown to stimulate (accelerate) the biosynthesis of ascorbic acid from D-glucorolactone by an enzyme preparation from goat liver extract.<sup>43</sup>

The conversion of L-gulonolactone into L-ascorbic acid could be affected by the above enzyme with or without the presence of  $CN^-$  but the conversion was greater in the absence of  $CN^-$ .

The  $CN^-$  mediated conversion has a high specificity requirement being possible only with the lactone form of the substrates, sodium salts of the free acids and other substrates being inactive.

From a study of enzyme inhibition it was postulated that SH groups of the enzyme may be involved in the  $CN^-$  mediated synthesis.

Roy and Guha<sup>44</sup> attempted to differentiate species according to their ability to synthesize ascorbic acid. It was concluded that among the amphibians and reptiles ascorbic acid synthesis took place in the kidney and their liver tissues could not accomplish such a synthesis. In majority of the species belonging to the natural order *Passeri formers* the site of ascorbic acid

synthesis was liver and not the kidney tissues.

### Citric Acid Synthesizing System

Deshpande and Ramakrishnan<sup>45</sup> have reported the presence of a condensing enzyme in the leaves of *Garcinia (Xanthochymus guttiferae)*. The enzyme preparation was shown to catalyse the synthesis of citrate from acetyl phosphate, Co A, and oxalacetate in the presence of a source of transacetylase. The authors claim that citric acid may be synthesized, at least in part, through the condensation of active acetate and oxalacetate.

Ramakrishnan<sup>46</sup> prepared a highly purified condensing enzyme from *Aspergillus niger* which appeared to be completely free from acetyl co-enzyme A deacylase. This enzyme preparation was shown to be inhibited by  $Mg^{++}$ , whereas acetyl co-enzyme A was activated by these ions. It is suggested that the condensing enzyme of *A. niger* may be different from that obtained from other sources in respect of  $Mg^{++}$  requirement.

During the course of their investigations on the mechanism of formation of citric acid in *Aspergillus niger*, Ramakrishnan and Raina<sup>47</sup> observed that during the early stages of fermentation of this mould no citric acid was formed despite the presence of several citrate synthesizing enzymes, such as the condensing enzyme, oxalacetic carboxylase, pyruvic oxidase, etc. Studies by these authors revealed the presence of two hydrolyzing enzymes oxalacetic hydrolase<sup>48</sup> and acetyl Co A deacylase.<sup>49</sup>

Oxalacetic carboxylase could be detected in 3 days old mycelium of *A. niger*. The enzyme activity was measured manometrically.

Acetyl Co A deacylase which hydrolyses the active acetate formed was present in the cell-free extract obtained from 3-day old mat of *A. niger*. The high activity of this enzyme in *A. niger* during early stages of incubation when no citric acid accumulates and its very low activity during later stages when citric acid accumulates suggest that during the early stages of fermentation this enzyme hydrolyses the acetyl Co A formed, thus preventing it from combining with the C<sub>4</sub> acid to form citrate.

### Flavokinase

Giri *et al.*<sup>50</sup> have reported on the occurrence of flavokinase activity in *Phaseolus radiatus*. There was no previously known plant source for this enzyme. The properties of the enzyme were studied by the application of circular paper chromatography. The optimum pH for the flavokinase activity was found to be 8.6, temperature 55°C, and conversion into flavine mononucleotide nearly 60%. A 75-fold purification was achieved by fractionation with ammonium sulphate and adsorption on alumina-C.

### Thiaminases

Giri<sup>51</sup> has reported upon the presence of two thiaminases in the fresh-water mussel, *Lamellidens marginalis*. These were separated by adsorption on alumina and elution with phosphate buffer pH 6.5.

### RNA—Depolymerase

Ribonucleic acid depolymerizing activity in certain cell-free preparations and resting cell suspensions of *Vibrio cholera* has been investigated by Sagar *et al.*<sup>52</sup> Intact cells have been reported to be entirely inactive; crude extracts obtained by grinding the cells with glass powder and extracting with 0.9 per cent potassium chloride solution were found to be highly active. More than 68 per cent of the activity was found associated with the supernatant obtained by centrifugation at 73,400 g. Depolymerization of RNA by cell-free extract is optimum at pH 8.0.

### FOLIC ACID CLEAVING ENZYME

Aravindakshan and Branganca<sup>53</sup> have reported that the ability of hydrolysed blood cells to cleave folic acid into p-amino-benzoyl-glutamic acid and 2-amino-4-hydroxy-6-formyl-pteridine can be enhanced by heating them to 100°C. for ten minutes. The active form of the enzyme liberated by thermal treatment has the same co-factor requirement as the ammonium sulphate fraction F<sub>2</sub>; Co++ acts as an activator and vitamin C as a potent inhibitor. The inhibition by the latter can be partially reversed by increasing the concentration of glutathione.

## ENZYME ACTIVATORS AND INHIBITORS, Etc.

Rao<sup>54</sup> has reported that desoxycorticosterone acetate and progesterone have good inhibitory effect on the amylase activity of human saliva; testosterone propionate has only a slight effect.

Srikantan *et al.*<sup>55</sup> have reported that cobalt, cysteine and 8-hydroxyquinoline activate aldolase of the cell extracts of eight virulent and avirulent strains of *P. pestis*. Ascorbic acid was found to be without effect. Of the antibiotics studied, neomycin and chloramphenical were found to have no action, whereas dihydrostreptomycin partially inhibited the enzyme.

Sohoni and co-workers<sup>56</sup> have reported that purified double bean trypsin inhibitor at the levels tested (0.265% level of casein diet) had no deleterious effect on the growth of the rats.

Sastray and Sarma<sup>57</sup> reported that intake of toxic amounts of zinc brings about a pronounced fall in tissue catalase activity. Addition of 0.01% copper sulphate to the zinc toxic diet restores the enzyme levels.

During a course of study of glucose metabolism in *S. griseus*, Rao<sup>58</sup> investigated the effect of inhibitors on the growth of *S. griseus*, streptomycin formation and glucose oxidation. Iodoacetate and arsenite were found to inhibit both growth and streptomycin formation. They also adversely affected the oxidation of glucose. Fluoride inhibits the growth slightly, has no significant effect on glucose oxidation, but increases the yield of streptomycin, presumably by inhibiting enolase, i.e., the C<sub>3</sub> fragments from glucose break-down are diverted into streptomycin biosynthesis.

### Miscellaneous

Chaudhuri *et al.*<sup>59</sup> have demonstrated histochemically the presence of tyrosinase activity in non-irradiated albino skins.

Shukla and Prabhu<sup>60</sup> have reported on the effect of ethylene diamine tetraacetic acid on the enzyme system of yeast cells.

Deshpande and Ganapathi<sup>61</sup> indirectly refer to the enzyme systems involved in the utilization of carbohydrates during biosynthesis of benzyl penicillin by resting cells of *P. chrysogenum*.

George and Eapen<sup>62</sup> have reported the presence of a number of enzymes in the visceral adipose tissue of pigeon: lipase, alkaline phosphatase and dehydrogenase, etc.

Rajagopalan and Sarma<sup>63</sup> have reported that the co-enzyme A content of *S. cerevisiae* cells grown in the presence of sulfanilamide was significantly greater than in normal cells.

In course of their investigations on the enzyme make up of *Salmonella typhosa*, Ghatak and Shrivastava<sup>64</sup> screened five antigenic strains of *S. typhosa* for aldolase activity, using both cell suspensions and cell free extract. Maximum activity was noted in strain H-901 and minimum in O-901. The optimum pH and substrate concentration for all the strains were 8.5 and 0.03 M respectively. Tetracycline and oxytetracycline inhibited the enzyme whereas chlortetracycline gave considerable activation. Co<sup>++</sup>, Zn<sup>++</sup> and cysteine activated the enzyme by 225, 75 and 600% respectively, whereas Mn<sup>++</sup> and Ni<sup>++</sup> proved to be inhibitors. A few more activators and inhibitors are described.

Mukherjee and Sarkar<sup>65</sup> studied liver enzymes in human under-nutrition.

Sachdev and Gupta<sup>66</sup> investigated the preventive action of the anti-fatty liver fractions of pancreas on fatty livers induced by ethionine. They reported that trypsin administered with casein in pancreatic duct-ligated rats prevented the accumulation of liver fat induced by ethionine. They suggested that the anti-fatty liver fraction of pancreas may have proteolytic activity.

Tulpule<sup>67</sup> reported that DPN deficiency, induced by protein or choline deficiency, decreased malic oxidase and DPNH-cytochrome C reductase reactivity in rat liver; both the apoenzymes and co-enzymes were adversely affected.

It was reported by Tulpule and Kshirsagar<sup>68</sup> that feeding of low proteins or pyridoxine deficient diets resulted in a decrease of blood glutamate-oxaloacetate transaminase (GOT) concentration in rats. Addition of missing nutrients to the diet, restored the GOT activity to normal in 2-3 weeks.

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## BIOCHEMICAL TECHNIQUES

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The subject of biochemical techniques was last reviewed for this publication by V. M. Sivaramakrishnan and P. S. Sarma<sup>1</sup> in 1954. The present account covers the studies relating to the same subject carried out in India from 1954 to 1958. The "Biochemical and Allied Research in India" fulfills a constructive purpose by annually supplying in a comprehensive manner an easily accessible survey of the research carried out in India, on the various topics of biochemical interest. With this object in view, therefore, an attempt has been made to include as many relevant investigations on the subject, published in recognised Indian and international journals, as are available to us. In the absence of an objective criterion, however, in order to abstract all work done in India, it is possible that some important studies may have been overlooked, for which apologies are extended.

During the years covered by the review, the technique of paper chromatography has been the most popular in India. This is to be expected in view of its simplicity and wide application. Although no significant contributions relating to the principles of the method of paper chromatography have been made, many studies have described innovations of a practical nature in its application to biological materials of importance in India. The late Prof. Giri and his associates have extensively developed the technique of circular chromatography and also used it, in a preparative scale. Among other interesting studies may be mentioned those of Jain who, by making use of a pH gradient, has been able to separate compounds with very closely related structures. Agar electrophoresis and paper electrophoresis have come into use in India during the last three years and these again have been popularised by Prof. Giri and his colleagues.

## Chromatography

In order to apply paper chromatography in a preparative scale to separate components from mixtures in milligram quantities, Giri<sup>2</sup> has adapted the chromatopile method originally described by Mitchell and Haskins<sup>3</sup> in 1949. This consists of a paper pack sandwiched between two glass plates which are placed over a basin in the centre of which a small beaker containing the solvent is kept. Solutions are spotted on the paper and a paper, wick, which passes through a hole made in the centre of the papers reaches the solvent in the beaker. The development is made several times by drying the paper each time. The bands are identified under the ultraviolet light. By the use of this technique, a mixture of ten sugars and also several sugars and amino acids have been separated. The solvent mixtures employed are described and several typical chromatograms are illustrated.

Narayanan and Menon<sup>4</sup> have constructed a chromatographic apparatus to attain uniform water-saturation and comparable partition coefficients. A strip of filter paper is spread horizontally over a parallel layer of moist cloth or similar other fabric, the whole arrangement being accommodated inside a one-litre cylinder held horizontally. The cloth is kept moist with the water-rich layer from an equilibrium mixture of butanol, alcohol and water. The mouth of the cylinder is closed with a rubber cork which has a bent glass tube passing through a hole in it. The authors claim that their values for partition coefficients of amino acids adhere better to the theory than those obtained by Consden *et al.*<sup>5</sup>

Subramanian and Rao<sup>6</sup> find better resolution and reproducibility of results obtained with the two dimensional chromatograms of amino acids if in the first phase phenol treated with pH 1.0 buffer is employed. This is claimed to prevent successfully the streaking of tyrosine and cystine. Quantitative recovery of added amino acids has been reported.

Jain<sup>7</sup> has developed an interesting method for the separation of haemoglobin components which is dependent on the pH gradient. The solutions are allowed to travel across a pH gradient, thus enabling the various types to become insoluble on the paper where suitable pH exists. The optimum time is reported to be

fifteen minutes only. It has been possible to separate successfully three components from normal haemoglobin all showing the same absorption spectra. The method may be used for the separation of compounds having closely related structures and different pK values.

Vasudevamurthy *et al.*<sup>8</sup> have studied the separation of inorganic ions by circular paper chromatography. The most effective solvent system is found to be a mixture of butanol, pyridine and water (10 : 2 : 2). The separation is effected in two hours and as such the method is considerably quicker than that reported by Lederer.<sup>9</sup>

Setty<sup>10</sup> has extended the above technique to analyse a greater variety of metal ions. His experience indicates that solvent systems containing acid are suitable for the purpose.

Ramachandran and Sarma<sup>11</sup> have successfully used ascending paper chromatographic technique coupled with the use of P,<sup>32</sup> to demonstrate that amylase contains phosphorus as an integral part of the protein. It is suggested that the phosphorus is present as a phospholipid moiety attached to the enzyme.

Lulla and Johar<sup>12</sup> have studied the sugar content of various kinds of bananas using n-butanol, acetic acid and water mixture as solvent and benzidine trichloracetic acid as spraying agent. The authors report the presence of maltose, sucrose, glucose and fructose among other carbohydrates.

Bhagwan and Rajagopalan<sup>13</sup> have studied the amino acid content of various kinds of bananas after acid hydrolysis. Cystine has been reported to be high in the types analysed.

Ramakrishnan<sup>14</sup> has been interested in the study of amino acid content of gaur flour. The chromatographic method of Rao and Wadhwani<sup>15</sup> has been used. Gaur flour was found to be rich in protein and about 17 amino acids have been detected. On germination the amino acid content was found to increase indicating that it may be a good quality protein.

Ravindranath *et al.*<sup>16</sup> have isolated the proteins of sesame seeds and studied their amino acid content. It is reported that the globulin fraction contains more of sulphur bearing amino acids than the whole meal.

Rao and Rao<sup>17</sup> have studied, by the two dimensional paper chromatographic method of Consden *et al.*<sup>5</sup> the amino acid changes occurring on tryptic digestion of heat denatured egg albumin. It is reported that arginine is the first amino acid released which is then followed by lysine, glutamic acid, tyrosine or tryptophan and leucine.

Sastray *et al.*<sup>18</sup> have separated polyphenols from various edible materials using sodium bisulphite in acetic acid as the solvent system and silver nitrate-ammonium hydroxide as the spraying agent. The presence of gallic acid, pyrogallol, catechol, apart from leucoanthocyanins, have been demonstrated.

Krishnamurthy and Swaminathan<sup>19</sup> have used several solvent systems, such as butanol, acetic acid water and phenol-butanol-acetic acid and water, and find good separation of arginine, threonine, alanine, tyrosine, methionine and phenylalanine by more than one solvent. This is a routine study which may be helpful as a ready reference for a suitable solvent convenient for the separation of a particular group of amino acid mixtures.

Ramakrishnan<sup>20</sup> reports the amino acid content of lipase separated from *Aspergillus niger*, using butanol-water-ethanol (20 : 4 : 5 : 1) and butanol-acetic acid-water solvent systems. The determination of lipase activity was carried out according to Ramakrishnan.<sup>21</sup> The presence of 13 different amino acids has been demonstrated.

Datta *et al.*<sup>22</sup> separately estimate methionine and valine in paper chromatograms. The separation is based on the fact that methionine is oxidised by hydrogen peroxide while valine is not. The estimation is done by determining the intensity of ninhydrin colour before and after exposure to hydrogen peroxide.

Giri<sup>23</sup> finds it convenient to convert tryptophan into a yellowish green fluorescent compound by treating the chromatogram with perchloric acid reagent, prepared by mixing perchloric acid and ethanol. The method can identify one microgram of tryptophan.

Bhattacharya *et al.*<sup>24</sup> report the quantitative estimation of histidine by paper chromatography. Histidine is separated from tyrosine and other imidazole compounds by unidimensional

chromatographic technique with butanol-acetic acid and water as the solvent system. The paper is dried and chromatographed by the method of Burma and Bannerjee.<sup>25</sup> It is then lightly sprayed with sodium carbonate solution and dipped into a diazo solution. The method gives a recovery of 98.5 to 103.5%.

Kumta *et al.*<sup>26,27</sup> have extended their earlier studies which indicated that aqueous solutions of methionine give a number of ninhydrin positive spots when irradiated with  $\beta$  and  $\gamma$  radiations from a radon needle. Methionine-S<sup>35</sup> and methionine-C<sup>14</sup>H<sub>3</sub> were irradiated to determine the types of products formed. The solutions after irradiation were subjected to two dimensional chromatography using n-butanol-acetic acid-water and then phenol water system in the second phase. The spots were radio-autographed and developed with ninhydrin. They detected the oxidation of methionine to methionine sulfoxide as well as the formation of homocysteine-like skeleton which finally gave rise to substances resembling threonine, aspartic acid and serine.

Sekharavarma and Ramakrishnan<sup>28</sup> have studied the organic acids in citrus plant tissues. The solvent system of Lugg *et al.*<sup>29</sup> was used. Citric, malic, and  $\alpha$ -keto glutaric and succinic acids are some of the acids that were detected.

Subbaram and Mahadevan<sup>30</sup> have quantitatively separated dicarboxylic acids from iso-oleic acid fraction of varaspati. They have employed columns of silica gel and a mixture of ethanol, methanol, water and benzene as the eluent. The isolation of undecanedioic, sebacic, azelaic and suberic acids is reported. The study has been further extended to the analysis of beef body fat and cow butter fat.<sup>31</sup>

Datta<sup>32</sup> reports the successful separation of cyanocobalamin from hydroxy cobalamin (vitamin B<sub>12</sub>a) in proteolysed liver extract by paper chromatography using a mixture of butanol, pyridine and water. Vitamin B<sub>12</sub> had a R<sub>f</sub> value of 0.24, and recovery of added vitamin B<sub>12</sub> has been found to be 98 per cent.

A mixture of thiamine and riboflavin has been separated by a method described by Giri and Balakrishnan.<sup>33</sup> The principle consists in converting thiamine to thiochrome and estimating the fluorescence under the ultraviolet light. The chromatogram is

developed and then treated with cyanogen bromide and alkali. Thiamine produces green, while riboflavin gives blue fluorescence under the above conditions. The method is apparently rapid, sensitive and suitable to estimate vitamin B<sub>2</sub> in multivitamin preparations.

Giri and Krishnaswamy,<sup>34</sup> while studying riboflavin metabolism in mutant BY<sub>2</sub> yeast, have developed a chromatographic method to separate FMN, FAD, lumiflavin and lumichrome. It is pointed out that circular paper chromatography leads to higher resolving power than the vertical type.

Iyer<sup>35</sup> has studied by paper chromatography the amino acid composition of red cells under normal and in hypochromic microcytic anaemic condition. Alanine, glutamic acid, glycine and serine were found to be elevated under the pathological state investigated. There was no correlation between the severity of the state and the increase in amino acid. This type of study has been extended to other pathological conditions also.<sup>36,37</sup> Various differences in the amino acid contents from normal have been reported without any attempt at interpretation of results.

Giri<sup>38</sup> has employed circular paper chromatography for the separation of serum proteins. It is reported that there is significant increase in the globulin fraction of blood serum in liver cirrhosis. The illustrations presented, however, do not give sufficient confidence so as to make the technique suitable for diagnostic purposes.

Padmavathy *et al.*<sup>39</sup> have detected glucose and fructose in the cerebro-spinal fluid, applying paper chromatography.

Wadia *et al.*<sup>40</sup> have successfully separated some easily oxidisable substances like sulphoxides and sulphones employed as anti-mycobacterial agents. The technique of paper chromatography employing the following solvent mixtures was found useful for the purpose; benzene-acetic acid, benzene-formic acid and butanol-ammonium hydroxide. The chromatograms were sprayed with 0.1 N HCl followed by sodium nitrite, and colour developed with n-sulphato ethyl-m-toludine.

Varma and Das<sup>41</sup> have carried out extensive and detailed chromatographic studies on the separation of a variety of dyes used in colouring foodstuffs. Numerous solvents useful for the

purpose and the  $R_f$  values obtained for more than 45 dyes have been listed. This is a useful reference-paper for those interested in the separation of dyes by paper chromatography.

Shrivastava *et al.*<sup>42</sup> have used hexane, dioxane and ammonium hydroxide mixture and also ethyl acetate, benzene and acetic acid mixture as solvents to separate reserpine from 3-epireserpine. The technique was applied to the study of reserpine metabolism *in vivo* in rats. The results showed the absence of 3-epireserpine in the urine of rats given reserpine.

Sarada Subramanian<sup>43</sup> has isolated isopropyl-nor adrenaline from human urine. A modified method of Vogt<sup>44</sup> was applied using phenol containing hydrochloric acid as solvent system. The spraying agent was 2 per cent potassium iodate instead of potassium ferricyanide as used by Vogt. The reference sample of isopropyl-nor adrenaline and the spot from urine gave the same  $R_f$  value of 0.69.

The problem of desalting of biological materials for chromatography has been overcome by the use of alcohol for the extraction of amino acids. Baliga *et al.*<sup>45</sup> concentrate the material under reduced pressure and dissolve the residue in 95 per cent alcohol in which the salts are not soluble.  $100 \pm 10$  per cent recoveries were obtained with added amino acids.

### Electrophoresis

Karkun and Dhar<sup>46</sup> have resolved intermedin (the hormone of the pars intermedia of the pituitary) into several fractions by electrophoresis. By this technique, they have separated intermedia from the contaminating ACTH. The electrophoresis has been carried out using Whatman No. 3 filter paper and barbiturate buffer pH 8.6. The hormone is resolved into five fractions of which fractions 2, 4 and 5 are active.

Mulgaonkar and Srinivasan<sup>47</sup> have studied the vitamin B<sub>12</sub> combining capacity of serum proteins. The serum proteins have been separated by paper electrophoresis. It has been found that most of the vitamin B<sub>12</sub> complexes with  $\alpha$  2-globulin. Serum  $\gamma$  globulin has been found not to combine with the vitamin.

Agar electrophoresis has been extensively employed by Giri

and others in the study of various protein fractions. Giri *et al.*<sup>48</sup> claim that results obtained for the analysis of sesame proteins compare well with those obtained with moving boundary electrophoresis.

The study of haemoglobin components of blood haemolysates of various species of animals by agar electrophoresis has been carried out by Giri and Pillai.<sup>49</sup> It has led to the successful separation of two haemoglobins from buffalo blood haemolysate.

In further studies Giri<sup>50</sup> has, with advantage, used agar electrophoresis to study serum proteins under various pathological states. An increase in the  $\beta$  and  $\gamma$  globulins of serum proteins of the blood in infantile biliary cirrhosis and Kala azar has been reported. After electrophoretic run at pH 8.6 for four hours, the proteins are made visible by treatment with 10 per cent TCA or by staining with naphthalene black 12B 200. The author is of the opinion that agar electrophoresis is better than the paper in so far as it offers less resistance to the movement of proteins. He suggests that it is more useful for the separation of enzymes and other biological constituents than paper electrophoresis.

The agar technique has been adapted by Giri<sup>51</sup> for use in tracer studies by forming the agar slab on cellophane or polyester film spread on a glass plate.

Sanghvi *et al.*<sup>52</sup> have found haemoglobin J in two Indian women. They have used paper electrophoresis as described by Lehmann and Smith<sup>53</sup> and starch electrophoresis according to the method of Kunkel.<sup>54</sup>

Study of sickle cell haemoglobin-S has been carried out in the blood of some tribal people of Western India by Sukumaran *et al.*<sup>55</sup> Among the nine tribes investigated, four were found to be positive. Percentage incidence varied from 9 to 22.

### Miscellaneous

Krishnamurthy and Swaminathan<sup>56</sup> have described a method for the determination of butylated hydroxy anisoles in fats. The principle of the method is based on the oxidation of the compound by ferric sulphate and the colorimetric estimation of the blue

complex formed between the ferrous sulphate so produced and added ferricyanide. The method gives values comparable with those obtained by the  $\alpha$ - $\alpha$  bipyridyl method.

Roy *et al.*<sup>57</sup> have reported a rapid method for the determination of total adrenal cholesterol. It is based on the principle that a stable purple colour, which obeys Beer's law is formed when a mixture of concentrated sulphuric acid, glacial acetic acid and ferric chloride is added to cholesterol in acetic acid. The authors claim values in agreement with those obtained by the standard Lieberman and Burchard reaction.

Nair and Magar<sup>58</sup> estimate vitamin E in serum or plasma by the formation of a coloured complex between vitamin E and phosphomolybdic reagent. The complex has maximum absorption at 725 m $\mu$ . The method is reported to detect 2 to 5 micrograms of vitamin E with recoveries of 98 per cent of added vitamin E.

Sovah Janah *et al.*<sup>59</sup> have modified the method of estimation of choline esterase by Huerga *et al.*<sup>60</sup>. The complex formed among the ferric ion, alkaline hydroxylamine and O-acyl derivatives is estimated. The assay is carried out before and after incubation with red blood cells as a source of enzyme. The authors failed to find any correlation between the cholinesterase of the erythrocytes and the degree of anaemia.

Desikachar *et al.*<sup>61</sup> have developed a method to detect adulteration in ghee using the turbidity temperature as the index. The method is based on the fact that when vanaspati is mixed with ghee, the turbidity temperature (the temperature at which clear liquid ghee becomes turbid) is elevated. The method is claimed to be easy and quick and able to detect adulteration at 20 per cent level.

Jagannathan *et al.*<sup>62</sup> have assayed aldolase by a spectrophotometric method. Aldolase and fructose diphosphate are mixed together in presence of small amounts of hydrazine, and the rate of increase in optical density at 240 m $\mu$  is found to be proportional to the enzyme concentration. The method is reported to be comparable in sensitivity to that of Warburg and Christian.<sup>63</sup> The new method has the advantage that the estimation can be carried out in presence of enzymes which oxidise or reduce DPN.

Sathe *et al.*<sup>64</sup> find that the spectrophotometric method described by Reddy and Srinivasan<sup>65</sup> cannot be used to determine microquantities of menadione and so have suggested modifications to suit the purpose. The method consists in treating menadione in ethyl alcohol with 2 : 4 dinitrophenyl hydrazine in 2N hydrochloric acid and incubating at 35°C for 10 minutes. The solution is treated with sodium carbonate and shaken and the colour is read at 635 m $\mu$  after extraction with amyl alcohol. Recovery experiments of added menadione give values ranging from 99.2 to 100 per cent.

Ramiah *et al.*<sup>66</sup> have estimated calcium in sugar-cane juice by a spectrophotometric method. This is dependent on a decrease in optical density at 630 m $\mu$  when EDTA is mixed with Ca++ and Cu++ in ammoniacal solution. It is claimed that the method gives values comparable with those obtained by the oxalate method and that it is suitable for routine use when large number of estimations have to be carried out.

Sampath Kumar *et al.*<sup>67</sup> have described a method for the preparation of dephosphocasein by treatment of casein with 0.05 N barium hydroxide. This dephosphocasein has been reported to be readily digested by proteolytic enzymes. It is claimed that this method has advantages over the usual preparation as it gives better yield, is quicker and does not lead to liberation of terminal amino acids.

Nerurkar and Sahasrabudhe<sup>68</sup> have modified the method of Holtz and Seekles<sup>69</sup> for the estimation of calcium by titration with EDTA. Ammonium purpurate in alkaline solution is used as indicator. The method has been reported to yield good recoveries and also results in agreement with those obtained by the method of Clark and Collip.<sup>70</sup>

Lulla and Johar<sup>71</sup> have described a method for large scale production of amylase by the open fermentation process using *Aspergillus oryzae*. Wheat bran extract medium is employed for the purpose. 2 c. c. formalin and 40 mg. penicillin G are found to keep off a contaminating rod shaped bacterium from the medium.

Hyaluronic acid has been prepared by Singh<sup>72</sup> from human umbilical cords by enzymic digestion, precipitation of polysaccha-

ride followed by adsorption and elution from ion exchange resins. The prepared hyaluronic acid is reported to be pure.

Ramachandran<sup>73</sup> has prepared arginine hydrochloride by a method which is the modification of the one reported by Pratt.<sup>74</sup> A comparative study of the two methods has been carried out and it is shown that the modifications introduced, increases the yield from 25 to 88 per cent.

Narurkar and Sahasrabudhe<sup>75</sup> have suggested a new method for the 'hydrolysis' of nucleic acids. The principle consists of dehydrating the sugar phosphate linkages by treatment with phosphorus pentoxide which effects the breakdown of the ribose bridge. Added purines and pyrimidines are reported to give good recoveries thereby showing that these components do not suffer degradation. In a subsequent paper,<sup>76</sup> the values obtained by the phosphorus pentoxide method are compared with those obtained by the standard perchloric acid method and shown to be in good agreement.

De<sup>77</sup> has estimated vitamin A by a fluorometric method using a highly sensitive fluorometer. The material is saponified with alcoholic potash and the unsaponified portion is extracted with petroleum ether. The estimation is done under ultra-violet using a suitable filter. The method is reported to give comparable values with the usual colorimetric and spectrophotometric methods. Vitamin D, carotene and cholesterol do not interfere with the estimation. The advantage of this procedure over the known methods is not very clear.

Damodaran *et al.*<sup>78</sup> have prepared uniformly labeled C<sup>14</sup>-glucose and fructose by photosynthesis. It is claimed that the method is simple. The isolation of glucose and fructose of high specific activities without the use of carriers has been worked out.

Bhattacharya and Kulkarni<sup>79</sup> have described a biosynthetic method to prepare C<sup>14</sup>-formate. Formate adapted *E. coli* cultures are grown in presence of C<sup>14</sup>O<sub>2</sub>. The formic hydrogenlyase mediates the reaction between CO<sub>2</sub> and hydrogen to form C<sup>14</sup>-formate. The special device required has been described in detail.

Gurnani *et al.*<sup>80</sup> describe the hydrolysis of proteins by 85 per cent formic acid. This treatment with formic acid reduces the

time of hydrolysis from 20 hours (by the usual method) to 2 hours. The release of amino acids by the two methods has been studied and found to be in agreement. Recoveries of added amino acids indicate that the amino acids are not destroyed during the process.

Braganca *et al.*<sup>81</sup> have separated 2-amino-4-hydroxy-6-formyl-pteridine from folic acid by adsorption and elution from ion exchange resin. The 6-aldehyde may then be identified by its characteristic absorption spectra. The method has been employed for the identification of the products formed in the oxidative cleavage of folic acid by blood. The method may be useful also in the identification of 6-aldehyde as a contaminant in commercial preparations of folic acid.

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## CHEMISTRY OF PLANT PRODUCTS

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During the year 1958, investigations on the chemistry of plant products have been pursued in various laboratories in India, though the tempo seems to have diminished somewhat, compared to the preceding year. A brief account of this work published in leading journals in India and abroad during 1958 is presented here. Articles of purely biochemical interest and those concerned with commercial and industrial aspects have not been included in this survey.

### Amino acids and proteins

The niacinogens isolated from both rice and wheat bran on hydrolysis yield <sup>1</sup> the same mixture of amino acids, viz., aspartic acid, glutamic acid, serine, glycine, threonine, alanine, tyrosine, leucine, isoleucine, phenylalanine, proline, arginine, histidine, lysine, cystine, methionine and valine. From the proportions in which these amino acids are present in the two niacinogens it appears that they are closely allied but not identical.

The leaves of *Lasiosiphon eriocephalus* have been shown to contain no less than eighteen amino acids. In addition, glucose, rhamnose, raffinose, sucrose, maltose and malic, citric, oxalic, malonic, fumaric and succinic acids have been shown to be present.<sup>2</sup>

The seeds of *Sterculia urens* contain an albumin, a glutenin and a globulin, the first two in very small quantities. The amino acid composition of the globulin has been determined,<sup>3</sup> cystine, lysine, histidine, arginine, asparagine, aspartic acid, glycine, serine, glutamic acid, threonine, alanine, tyrosine, phenylalanine, valine, leucine, isoleucine and tryptophan being present.

A procedure for the synthesis of dl-serine has been described.<sup>4</sup>

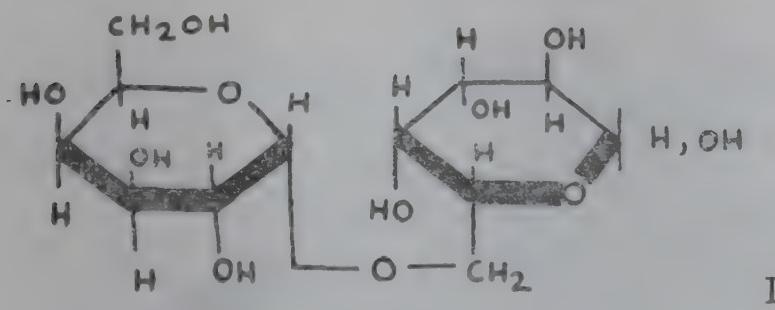
## Carbohydrates and glycosides

Fructose in sugar-cane is utilised more during the rapid vegetative, initiative and short blade stages, while it is in lesser demand at arrowing stage.<sup>5</sup> High sucrose content is followed by a rapid decrease in hexose sugars and a very high fructose / glucose ratio.

*Sesbania grandiflora* seeds show evidence for the presence of more ascorbic acid at comparable periods of germination than other legumes, with the exception of green gram.<sup>6</sup>

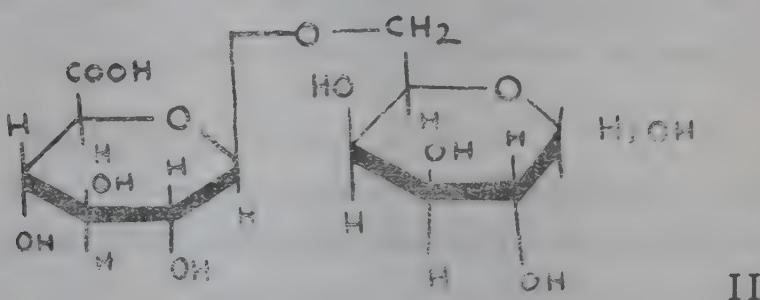
Eight cereal and eight legume starches have been analysed for amylose and amylopectin content potentiometrically and spectrophotometrically.<sup>7</sup>

Swietenose,<sup>8</sup> a new disaccharide isolated from Gum Mokha (*Schrebera swietenoides*) on complete methylation by dimethyl sulphate and alkali and Purdie's reagent followed by hydrolysis yielded a mixture of 2 : 3 : 4 : 6 tetra-O-methyl-D-galactose and 2 : 3 : 4-tri-O-methyl-D-galactose. Swietenose consumed six moles of periodic acid with liberation of five moles of formic acid. It is therefore formulated<sup>9</sup> as 6-O- $\alpha$ -galactopyranosido-D-galactoside (I).



Acacia sundra gum yields on hydrolysis D-galactose, L-arabinose, L-rhamnose, and D-glucuronic acid. The aldobioronic acid component of the gum obtained by graded hydrolysis after methylation and hydrolysis yielded equal amounts of 2 : 3 : 4-tri-O-methyl D-galactose and 2 : 3 : 4-tri-O-methyl D-glucuronic acid. The aldobioronic acid was laevorotatory. On these grounds it has been

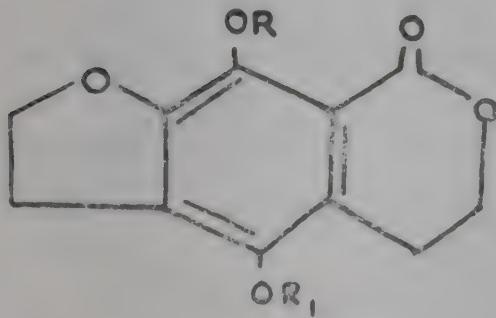
assigned<sup>10</sup> the structure 6-O-( $\beta$ -D-glucopyranosyluronic acid)-D-galactose. (II).



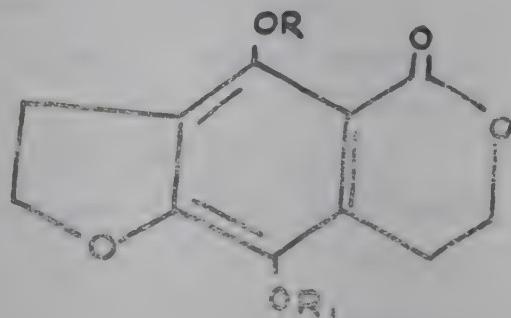
Two apparently new glycosides, peruvoside,  $C_{30}H_{46}O_{10}$ , m.p., 160-164°/210-216°,  $[\alpha]_D^{29} = -69.6^\circ$  and ruvoside,  $C_{30}H_{46}O_{10}$ , m.p., 228-230°,  $[\alpha]_D^{29} = -45^\circ$ , have been isolated<sup>11</sup> from the seed kernels of *Thevetia nerifolia*, besides the known glycosides cereberin, nerifolin and thevetin. Both the new glycosides gave thevetose on hydrolysis. The aglycone from peruvoside was amorphous, while the aglycone fraction from ruvoside gave two crystalline compounds, m.p., 226-228° and 168-170° respectively. Three new crystalline glycosides, m.p. 245°, m.p., 224° and m.p. 265-270° have been isolated<sup>12</sup> from *Cicer arietanum* (Chana).

Blepherin,<sup>13</sup> the glucoside isolated from *Blepharis edulis* has been investigated.<sup>14</sup> The glucoside is not methylated by diazomethane, but only by dimethyl sulphate and alkali, indicating the presence of a bounded hydroxyl group. Blepherigenin, the corresponding aglucone yields a monomethyl ether different from that obtained on hydrolysis of blepherin monomethyl ether. Both the monomethyl ethers yield the same dimethyl ether on further methylation. Blepherigenin shows infrared absorption characteristic of an  $\alpha : \beta$ -unsaturated  $\delta$ -lactone and ultraviolet absorption similar to that of a dihydroisocoumarin. It is also reduced to a diol by lithium aluminium hydride. One oxygen atom is inert and has been suggested to be part of a dihydroturano ring. On these grounds blepherin has been formulated as 3:4-dihydro-5- $\beta$ .

D-glucosyloxy-9-hydroxy-(X)-dihydrofuran iso coumarin (III or IV).



III



IV



Proceranin, m.p., 156-158°, has been isolated<sup>15</sup> from *Albizzia procera*. The sapogenin has been identified<sup>16</sup> as machaerinic acid and the oligosaccharide has been shown to consist of D-glucose, D-arabinose, D-xylose and L-rhamnose.

Graveobioside B has been shown<sup>17</sup> to be a mixture of apolin and chrysoeriol-7-apiosylglucoside.

### Fats and oils

The fatty acid composition of the oil from the seeds of *Lantana camara var. aculeata* has been determined.<sup>18</sup> The component acids and fats of the seeds of *Myristica beddomei* have been investigated.<sup>19</sup> The major portion of kamala seed oil is composed of complex triglycerides formed by the condensation of various constituent fatty acids both with the hydroxyl groups of glycerol and of α-kamolenic acid. The rest of the oil, about 12% consists of simple triglycerides of the component fatty acid.<sup>20</sup>

The fatty acid composition of the seed fat of *Trichosanthes cucumerina* has been determined.<sup>21</sup> Notable features are the presence of arachidic acid and the large amount of conjugated triene acid, probably trichosanic acid.

Enrichment of elaeostearic acid has been achieved<sup>22</sup> by chromatography of a mixture of unsaturated fatty acids on silica and Hyflo Supercel.

Studies of the aeration of a number of natural fats, like ghee, lard, groundnut oil and sesame oil show that the natural fats have the same autoxidation pattern as the unsaturated fatty esters.<sup>23</sup>

The mobilisation of reserve fats in germinating seeds appears to proceed<sup>24</sup> in a non-selective manner, there being no preference for the utilisation of unsaturated fats as claimed by Ivanov.<sup>25</sup>

A procedure for the production of lactic acid from Mowrah flowers by alkali degradation and fermentation has been described.<sup>26</sup>

### Terpenoids

The proceedings of a symposium held at Dehra Dun in 1954 on different aspects of the essential oil industry have been published by the Council of Scientific and Industrial Research under the title 'Essential Oils & Aromatic Chemicals.'

The essential oil from the berries of *Laurus nobilis* contains cineole, alcohols (chiefly terpineol), esters (chiefly methyl cinnamate), cinnamic acid, phenols and terpenes (chiefly  $\alpha$ - and  $\beta$ -pinenes).<sup>27</sup>

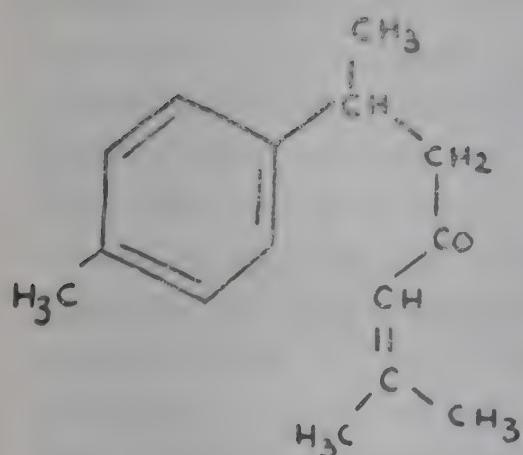
Analysis<sup>28</sup> of the essential oil from *Murraya koenigi* shows the presence of mono and bicyclic terpenes (29%) and sesquiterpenes (71%), with small quantities of higher fatty acids.

The essential oil from *Eucalyptus citriodora* (from Coonoor, Madras State) contains<sup>29</sup> over 82% of citronellol, unlike the oil from the normal form (Australia) which contains the aldehyde citronellal (25-80%).

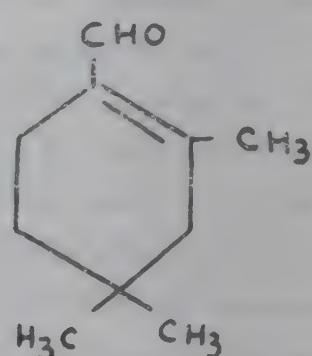
The oil from *Cymbopogon martini* var. *Sofia* has been analysed.<sup>30</sup> It contains nearly 54% of alcohols consisting of geraniol, nerol and dihydrocuminal alcohol and limonene, phellandrene, dipentene, citral and carvone in small quantities. Formic, acetic and butyric acids are also present.

The roots of *Angelica archangelica* from the hills of Gulmarg and Gurez in Kashmir, yield on steam distillation 0.55 % of an oil containing malic acid,  $\alpha$ -pinene, phellandrene, osthole, 15-hydroxy pentadecanoic acid, a solid m.p. 38-39° and appreciable amounts of terpenes and sesquiterpenes.<sup>31</sup> The essential oil from *Angelica glauca* has been analysed.<sup>32</sup>

A new synthesis of carvotanacetone has been reported.<sup>33</sup> A new synthesis of α-turmerone (V) has been effected<sup>34</sup>.



V



VI

Oppenauer oxidation of α- as well as β-cyclolavandulols gives β-cyclolavandulal. A new synthesis of the last compound has been achieved<sup>35</sup> in the following way: 3 : 3-dimethyl cyclohexanone was formylated with ethyl formate and sodium to give 3 : 3-dimethyl-6-hydroxymethylenecyclohexanone. This compound on treatment with isobutanol in boiling benzene was converted to the corresponding isobutoxymethylene derivative. Grignard reaction on this enol ether ketone followed by hydrolysis yielded β-cyclolavandulal (VI).

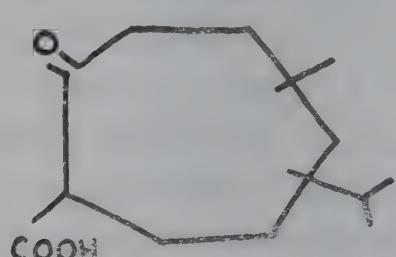
A mechanism for the formation of carvestrene dihydrochloride from fenchyl alcohol has been proposed.<sup>36</sup>

A study<sup>37</sup> of the perbenzoic acid oxidation of longifolene has shown that in controlled oxidations using one mole of perbenzoic acid, the isolable primary product is the aldehyde and not the epoxide. Further oxidation of the epimeric mixture of longifolaldehydes led to a mixture which was resolved by chromatography into longicamphenilone, the epimeric longicamphenilols, longidione, α-longifolic acid, α-longifloric acid acid and a liquid corresponding to the formates of the longicamphenilols.

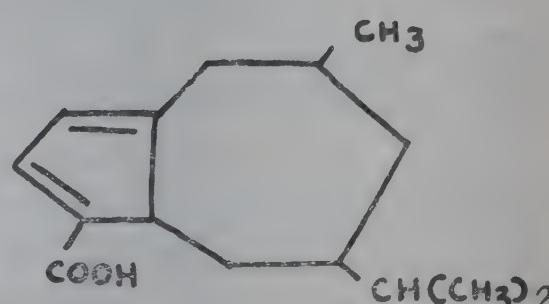
In a reinvestigation<sup>38</sup> of the essential oil from *Nardostachys jatamansi*, neither jatamansic acid<sup>39</sup> nor any carbonyl component could be isolated, but the isovaleryl ester of an alcohol, C<sub>15</sub>H<sub>24</sub>O and a hydrocarbon C<sub>15</sub>H<sub>24</sub> were the only components which

could be obtained. However, another group of workers has reported <sup>40</sup> the isolation in good yield of a ketone, jatamansone from the same essential oil. The ketone has been properly characterised and the corresponding alcohol has been shown to yield a new azulene on Pd-C dehydrogenation.

The structure of jatamansic acid has been investigated.<sup>41</sup> On ozonolysis, a keto acid C<sub>12</sub>H<sub>20</sub>O<sub>3</sub> was obtained. This is formulated as a  $\beta$ -keto acid (VII), surprising in view of the fact that it has been purified by distillation. On heating jatamansic acid with soda lime at 320° a greenish blue liquid was obtained, considered to be a 5:7-dialkylazulene. On the basis of these data and consideration of the ultraviolet and infrared absorption and nuclear magnetic resonance spectra jatamansic acid has been assigned structure (VIII).

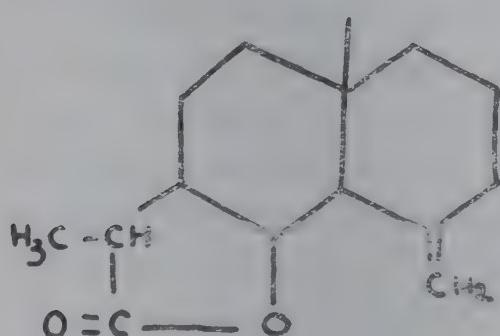


VII

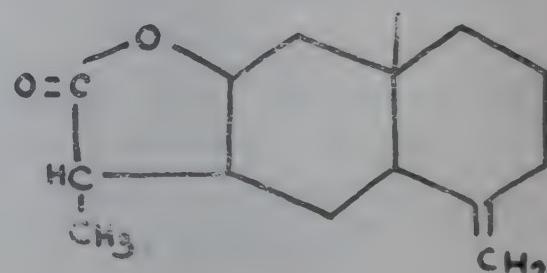


VIII

*Sassaurea lactone* from *Sassaurea lappa* has been found <sup>42</sup> to contain a terminal methylene group and  $\gamma$ -lactone system and to yield on selenium dehydrogenation 1-methyl-7-ethylnaphthalene. On these grounds it has been assigned the alternate structures (IX) and (X).

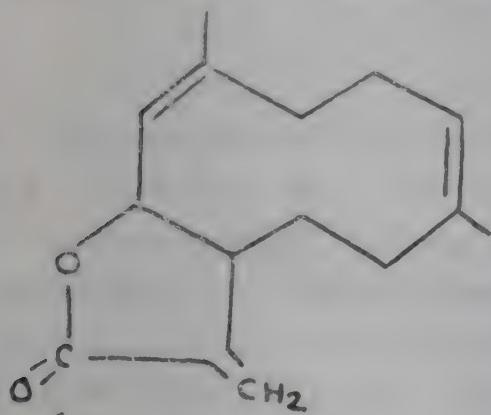


IX

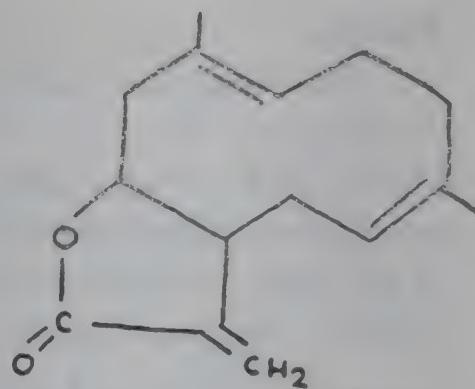


X

A new sesquiterpene lactone costunolide,  $C_{15}H_{29}O_2$  has been isolated<sup>43</sup> from costus oil (*Sassurea lappa*). This is considered to contain a ten-membered carbon ring, three double bonds, of which one is a terminal methylene group and an  $\alpha : \beta$ -unsaturated  $\gamma$ -lactone system. Ozonolysis yields formaldehyde and laevulinic acid. Costunolide has been formulated tentatively as either (XI) or (XII).



X



XI

A crystalline bitter principle cucurbitacin B,  $C_{28}H_{40}O_7$ , m.p. 182-185° and oleanolic acid have been isolated<sup>44</sup> from the seeds of *Luffa acutangula*. Oleanolic acid has also been isolated<sup>45</sup> from *Achyranthes aspera*. The saponin isolated from *Achyranthes aspera* has been characterised<sup>46</sup> as oleanolic acid oligosaccharide. The sugar moiety is composed of glucose, galactose, xylose and rhamnose.

Echinocystic acid  $C_{30}H_{48}O_4$ , m.p. 304-307° and albigenic acid,  $C_{30}H_{48}O_4$ , m.p. 233-236° have been isolated<sup>47</sup> from the beans of *Albizia lebbeck* by alcohol extraction. Lupeol has been isolated from the bark of *Emblica officinalis*. From the roots of *Coccinea indica*, lupeol,  $\beta$ -amyrin and  $\beta$ -sitosterol have been isolated.<sup>48</sup> *Exacum bicolor* was found<sup>49</sup> to yield ursolic acid. The same acid was obtained<sup>50</sup> also from *Oldenlandia biflora* and *Oldenlandia herbaceae*.

From the trunk bark of *Melia azadirachta*, besides nimbin, nimbinin and nimbosterol,<sup>51</sup> three other crystalline components have been isolated.<sup>52</sup> One, m.p. 292-294° was identical with

sugiol, the second nimbol, m.p. 244° also phenolic is apparently new and the third, a solid m.p. 82-83°, C<sub>26</sub>H<sub>54</sub>O, is probably a branched chain alcohol. Nimbosterol has been shown to be identical with  $\beta$ -sitosterol.

The observations made by Narasimhan<sup>53</sup> on the functional groups of nimbin have been largely confirmed.<sup>54</sup> Minor differences relate to interpretation of infrared spectra of nimbin and its degradation products.

### Steroids

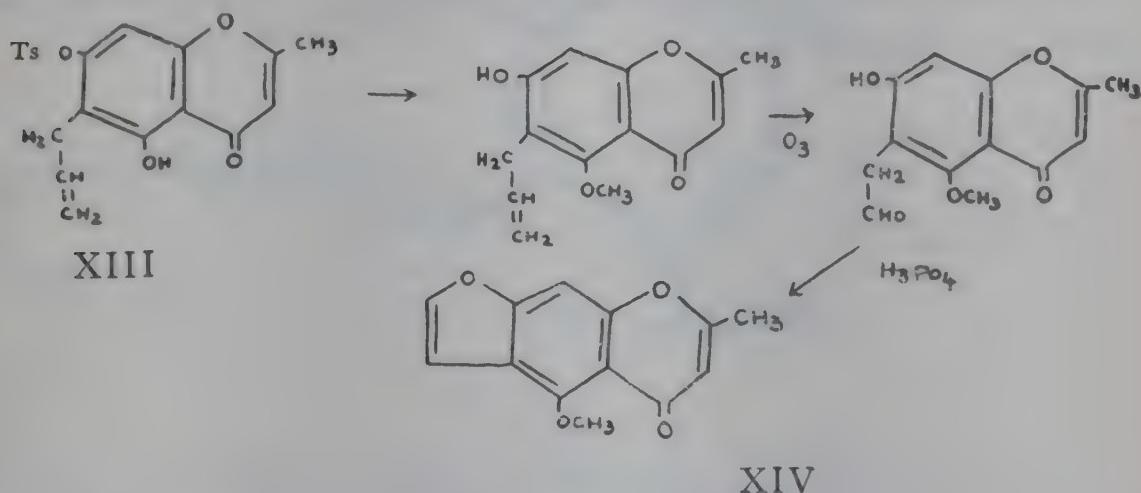
The sterol from *Aegele marmelos* has been identified<sup>55</sup> as  $\gamma$ -sitosterol. The same sterol has also been obtained<sup>56</sup> from chaksu oil from the seeds of *Cassia absus*. Examination of the whole plant of *Crotalaria medicagenia* showed<sup>57</sup> the presence of  $\beta$ -sitosterol, pentatriacontane and n-triacontanol, besides myristic, behenic and oleic acids. A sterol, m.p. 122°,  $[\alpha]_D^{30} = -57.9^\circ$ , forming a digitonide has been obtained<sup>58</sup> from *Bassia latifolia*. From *Convolvulus pluricaulis* two crystalline substances, C<sub>28</sub>H<sub>50</sub>O<sub>2</sub>, m.p. 124-125° and C<sub>40</sub>H<sub>60</sub>O<sub>5</sub> m.p., 64-65° stated to give colour reactions for sterols have been isolated.<sup>59</sup>

A simple and efficient method for the isolation of diosgenin from *Dioscorea prazeri* and *Dioscorea deltoidea* has been described.<sup>60</sup> A novel feature of this method is the carrying out of the hydrolysis and extraction of diosgenin in a single stage. A method for the assay of the diosgenin content of yams of *Dioscoreas* has been described.<sup>61</sup> Paper chromatographic procedures for the separation and identification of digitoxin, gitoxin and digoxin have been reported.<sup>62</sup>

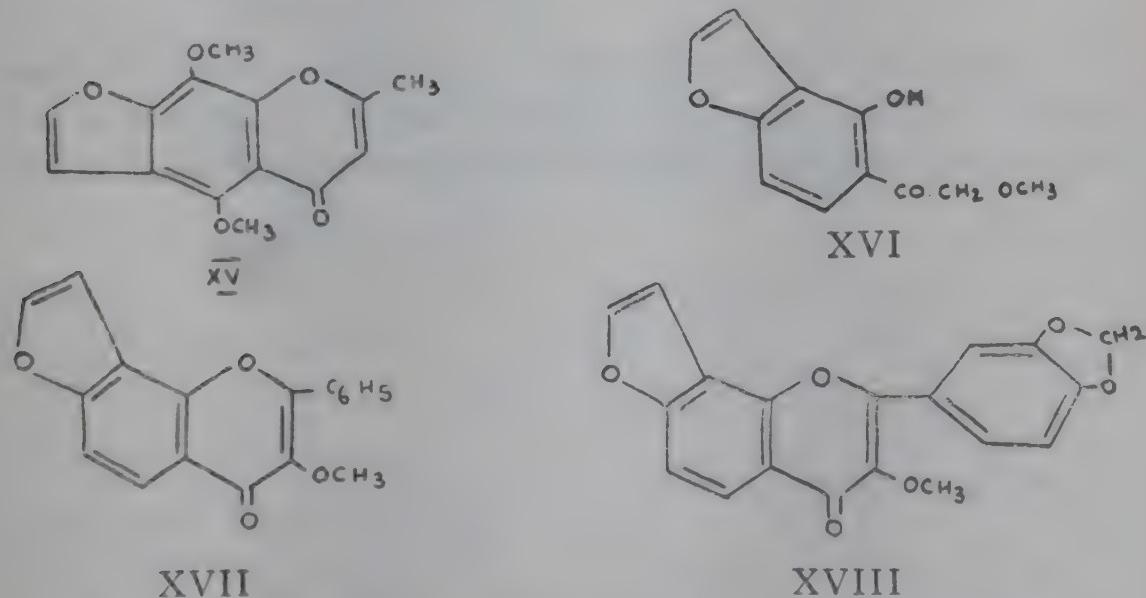
### Natural colouring matters and related compounds

Isoformonetin (7-methoxy-4'-hydroxyisoflavone) and daidzein (7 : 4'-dihydroxyisoflavone) have been synthesised<sup>63</sup> by a new route from 7-methoxy-4'-nitro- and 7-hydroxy-4'-nitroisoflavones respectively through the corresponding aminoisoflavones. Aqueous borax solution removes gossypol from an ether extract of cotton seeds and the gossypol could be precipitated by treatment with mineral acid. The product is obtained in good yield and is readily purified.<sup>64</sup>

A new method of preparing benzofurans using substituted allyl phenols has been worked out. Ozonolysis of the allyl derivative and subsequent ring closure of the resulting *o*-hydroxy-acetaldehydes by means of phosphoric acid leads to the benzofurans. For instance 2-methyl-5-hydroxy-6-allyl-7-tosyloxychromone (XIII) is methylated and detosylated to yield 2-methyl-5-methoxy-6-allyl-7-hydroxychromone. Ozonolysis and treatment of the resulting aldehyde with phosphoric acid gave<sup>66</sup> a good yield of visnagin (XIV).



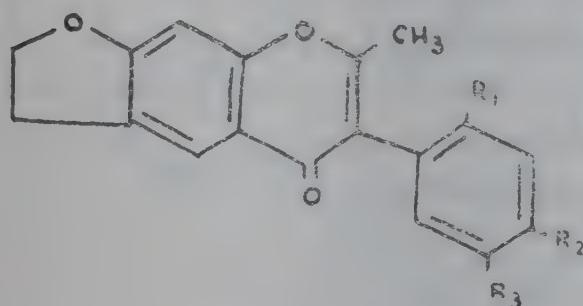
By the same method, khellin<sup>66</sup> (XV), karanjin ketone<sup>67</sup> (XVI), karanjin<sup>67</sup> (XVII) and pongapin<sup>67</sup> (XVIII) have been synthesised.



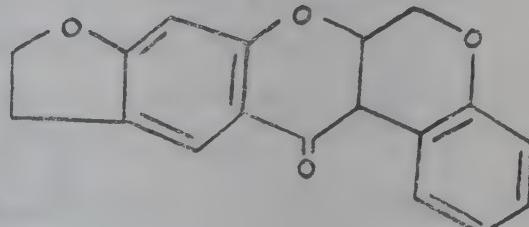
Another synthesis of karanjin ketone and karanjin has been effected.<sup>68</sup> Acetyl karanjoyl chloride was converted to the

diazoketone, which suffered decomposition in absolute methanolic medium in the presence of copper to yield 5 ( $\omega$ -methoxy)-acetyl-4-hydroxycoumarone, identical with karanjin ketone. The last compound on Allan-Robinson benzoylation yielded karanjin.

The syntheses of three 2-methyl dihydrofuranoisoflavones (XIX a, b, c) has been described.<sup>69</sup> A close structural analogue of pachyrrhizon, was prepared by the dehydrogenation of the corresponding dihydrofuranoisoflavone (XIX c). The synthesis of the dihydrofurano-(3":2"-6:7)-chromono-(2:3-3:4') chromene (XX) representing the five-ringed basic akeleton of pachyrrhizon has also been accomplished.



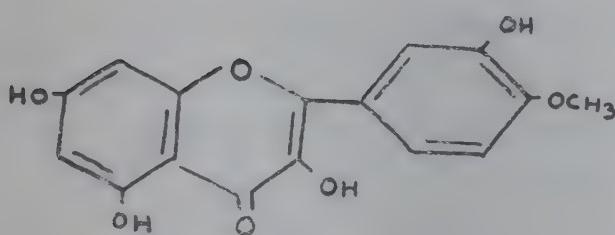
XIX



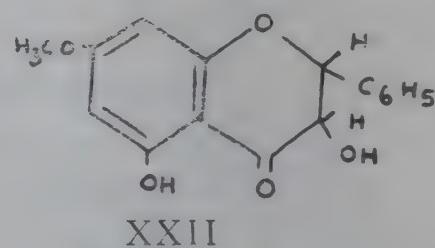
XX

- (a)  $R_1 = OCH_3$ ;  $R_2 = R_3 = H$ ;
- (b)  $R_1 = H$ ;  $R_2 = R_3 = OCH_3$ ;
- (c)  $R_1 = H$ ;  $R_2, R_3 = \begin{array}{c} -O \\ || \\ CH_2 \\ || \\ -O\end{array}$

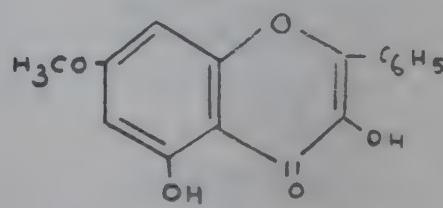
Simpler syntheses of tamaraxetin (XXI), alpinone (XXII) and izalpinin (XXIII) have been worked out.<sup>70</sup> In the synthesis of tamaraxetin, hesperetin, easily available from hesperidin, is convert-



XXI



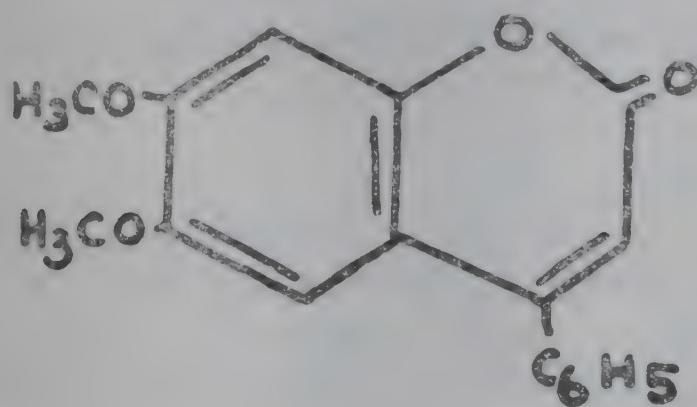
XXII



XXIII

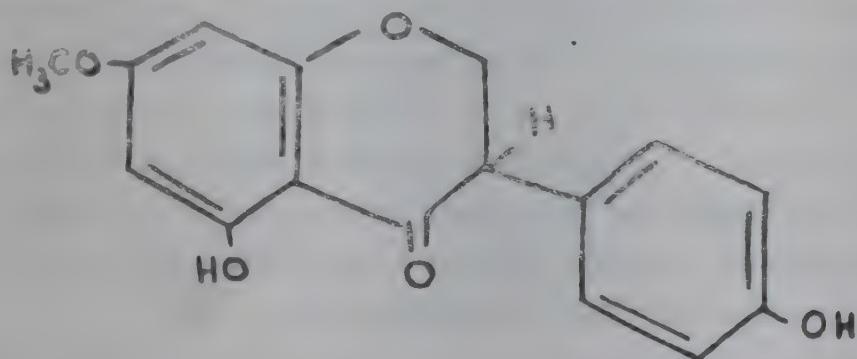
ed to 3-acetoxyhesperetin by iodine and silver acetate, which is then hydrolysed and dehydrogenated to yield tamaraxetin. 5-Hydroxy-7-methoxyflavanone (pinostrobin) has been converted to alpinone by Fenton's oxidation and subsequent dehydrogenation yielded izalpinin.

O-Methyldalbergin (XXIV) and related 4-phenylcoumarins yield coumarilic acids on boiling with alkali and mercuric oxide. The mechanism of this process has been discussed.<sup>71</sup>



XXIV

The synthesis of padmakastein (XXV) has been effected<sup>72</sup> by the catalytic reduction of prunetin diacetate and subsequent deacetylation. Similarly prunetin dimethyl ether on reduction yields padmakastein dimethyl ether.



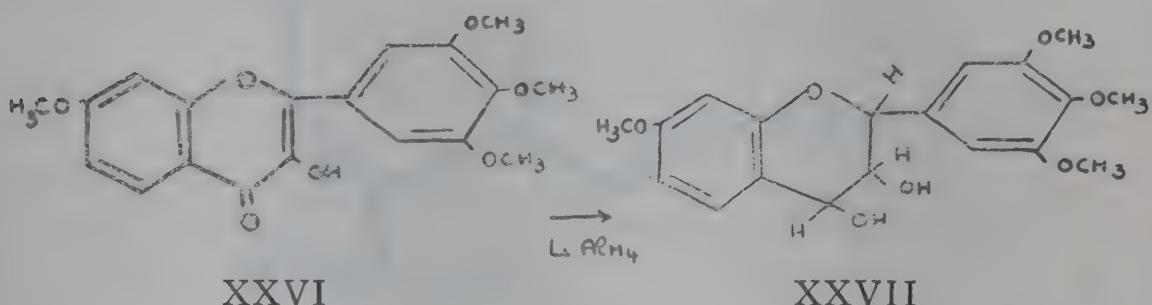
XXV

A study of the nuclear methylation of chalkones and flavanones has been made.<sup>72a</sup>

Feronialactone,  $C_{19}H_{23}O_3$ , m.p. 68-69° was isolated<sup>73</sup> from the root bark of *Feronia elephantum* by extraction with petroleum ether. Its ultraviolet absorption spectrum resembled tha

of a coumarin and its infrared absorption spectrum showed bands at  $1730\text{ cm}^{-1}$  ( $\alpha:\beta$ -unsaturated  $\delta$  lactone) and  $1122\text{ cm}^{-1}$  (ether). On pyrolysis it yielded 7-hydroxy coumarin and on ozonolysis acetone, laevulinic aldehyde and glyoxylic acid. It was therefore formulated as 7-geranyloxycoumarin. The structure was confirmed by synthesis.

The synthesis of dihydrorobinetin tetramethyl ether (XXVI) and its conversion to the corresponding leucoanthocyanidin (XXVII) by reduction with lithium aluminium hydride have been described.<sup>74</sup>



XXVII

From the alcoholic extract of tamarind seed testa, ethyl acetate-soluble and ethyl acetate-insoluble fractions were isolated.<sup>75</sup> The former was found to be leucocyanidin, stereoisomeric with that from *Butea* gum. The latter resembles leucocyanidin and has the same 2-phenyl side chain. A laevorotatory leucodelphinidin has been isolated from Karada bark (*Cleistanthus collinus*) while kino (gum) from *Eucalyptus pilularis* gave a dextrorotatory leucodelphinidin.<sup>76</sup> A third isomer with a lower dextro rotation is present in *Myrica megii*.<sup>77</sup> The bark of *Emblica officinalis* contains<sup>78</sup> a leucodelphinidin identical with that present in *Eucalyptus pilularis*. Leucopelargonidin has been isolated<sup>77</sup> from *Eucalyptus calophylla* and leucocyanidin from *Butea frondosa* gum.

In order to get information on the influence of hydroxyl groups on alkali colour reactions of flavonols, a number of flavonols of types uncommon in nature have been prepared and examined.<sup>79</sup> The relation between structure and colour reaction is complex and can only be of limited use for diagnostic purposes.

The possibility of various essentially non-terpenoid compounds containing isoprene units, being derived from a hypothetical

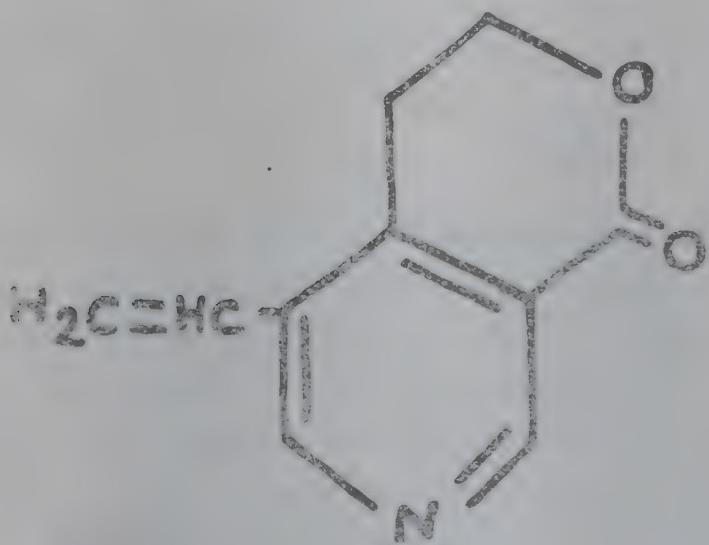
aldehyde  $\text{H}_2\text{C}-\overset{\text{CH}_3}{\underset{\text{COOH}}{|}\text{C}}=\text{CH}-\text{CHO}$  is discussed.<sup>80</sup> In his

Presidential address to the Indian Chemical Society Bose has given an interesting account<sup>81</sup> of the biochemical properties of a number of naturally-occurring coumarins.

### Alkaloids

Comprehensive reviews have been published on the Kurchi alkaloids<sup>82</sup> and on the biosynthesis of alkaloids in plants.<sup>83</sup>

The co-occurrence in nature of conhydrine and *pseudo*-conhydrine which are derivatives of coniine carrying a hydroxyl group at a  $\beta$  position to the nitrogen, appears to suggest according to Robinson, a kind of oriented oxidation.<sup>84</sup> A laboratory analogy has been furnished<sup>85</sup> by carrying out the rearrangement of 2-n-propylpyridine-1-oxide, yielding 2-1'-hydroxypropyl-pyridine (49%) and 5-hydroxy-2-propylpyridine (2.5%).



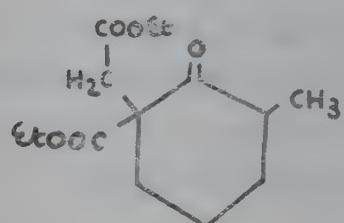
XXVIII

A biosynthetic scheme for gentianine (XXVIII) has been proposed<sup>86</sup> in which the key step is 'Woodward fission'<sup>87</sup> of a 6:7-dihydroxyisoquinoline precursor.

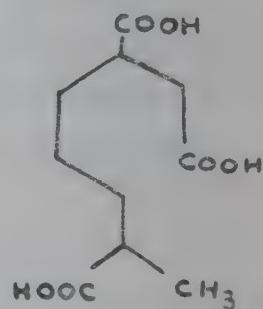
Chaksine nitrate was stated<sup>88</sup> to undergo decomposition on treatment with concentrated sulphuric acid, yielding two compounds, one a sulphate  $(\text{C}_{11}\text{H}_{17}\text{N}_4\text{O}_3)_2\text{SO}_4$  and the other a base

$C_8H_{13}N_3O_3$ . The products obtained have now been shown<sup>89</sup> to be nitrochaksine sulphate and nitrochaksine respectively. The action of nitrous acid on chaksine chloride leads to chaksine nitrate.

Chaksinic acid, obtained by the alkaline hydrolysis of chaksine iodide has been synthesised. 2-methyl-6-carbethoxy-cyclohexanone was alkylated by ethyl bromoacetate in the presence of potassium tertiary butoxide to yield 2-methyl-6-carbethoxycyclohexanone-6-ethyl acetate (XIX). Ring opening by means of sodium ethoxide in boiling absolute ethanol followed by alkaline hydrolysis resulted in the formation of 1:2:6-heptanetricarboxylic acid, which was identical with chaksinic acid (XXX).



XXIX

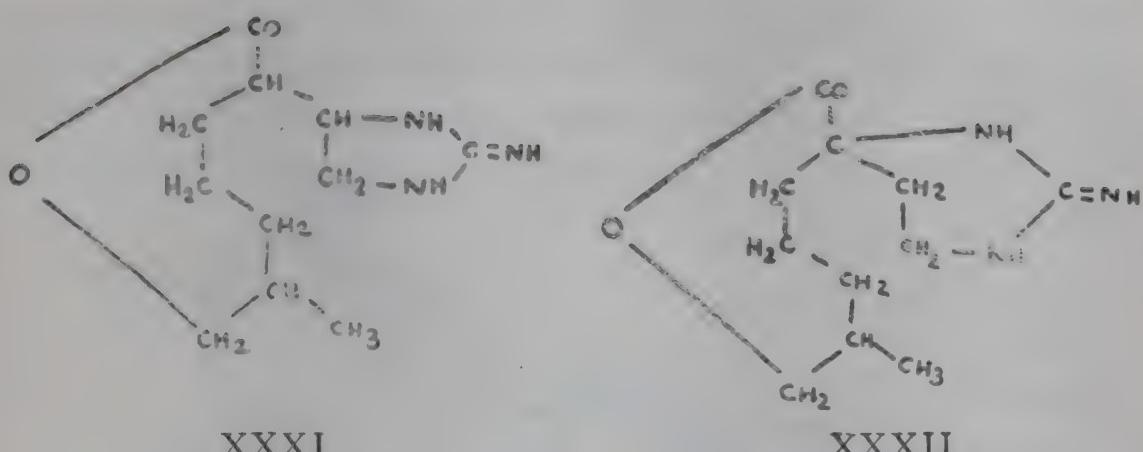


XXX

In the course of establishing the structure of chaksinic acid, four other structural isomers were also synthesised.

The presence of two bands at  $5.81 \mu$  and  $5.98 \mu$  in chaksine iodide were ascribed to the  $> C = N -$  function of the guanidino part and to the presence of a large-membered lactone ring respectively. From a consideration of the molecular formula of chaksine  $C_{11}H_{19}O_2N_3$  and the functional groups present, it is concluded that chaksine must be bicyclic.<sup>91</sup> Independently of the Indian workers a structure (XXXI) has been suggested for chaksine by Wiesner and co-workers<sup>92</sup> in Canada. The Indian workers however think that an alternate structure (XXXII) is equally consistent with the degradation evidence.

A third structure has been proposed<sup>93</sup> for chaksine in which it is formulated as a  $\gamma$ -lactone with little justification and the conclusions of Wiesner *et al.* have been challenged.<sup>94</sup>



XXXI

XXXII

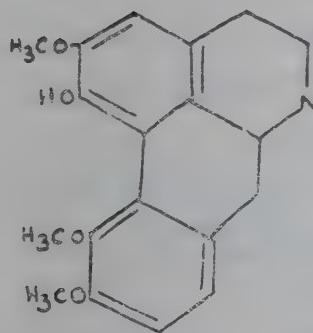
Full details about the elucidation of structure of aegelin, the alkaloid from *Aegele marmelos*, have been published.<sup>95</sup> The ultraviolet spectra of aegeline, aegelone, dihydroaegelin and dihydro-aegelone have been recorded. On the basis of ultraviolet absorption data, aegelin is confirmed to be a derivative of trans-cinnamic acid.<sup>96</sup> A modified method for the isolation of aegelin from the leaves of *Aegele marmelos* has been described.<sup>97</sup>

From *Evolvulus alsinoides*, a new alkaloid evolvine, C<sub>12</sub>H<sub>17</sub>O<sub>2</sub>N has been isolated and characterised.<sup>98</sup> Two methods for the estimation of total alkaloids in tincture of ephedra have been described.<sup>99</sup> In the first method, the alkaloids are extracted from concentrated tincture by dilute acid, the acid extract shaken with petroleum ether, saturated with sodium chloride, made alkaline with ammonia and shaken with ether to remove the alkaloids. The ether solution is evaporated and the alkaloidal residue dissolved in acid and estimated by titration against alkali. In the second method, the concentrated tincture is treated with lead acetate solution, the precipitate obtained filtered off, the excess lead precipitated by dilute sulphuric acid and the alkaloids present in the acid filtrate extracted and estimated as in the first method.

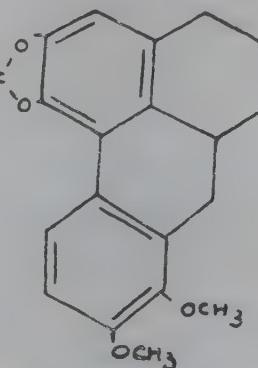
The structure of the phenolic aporphine alkaloid corydine (XXXIII) has been confirmed<sup>100</sup> by the synthesis of its racemic form.

The formulation<sup>101</sup> of crebanine as 1 : 2-dimethoxy 5 : 6-methylenedioxy aporphine was considered unsatisfactory by Manske<sup>102</sup> on biogenetic grounds. The correctness of this

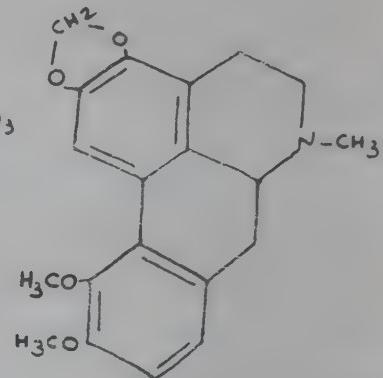
structure has been established<sup>103</sup> by a synthesis of dl-crebanine (XXXIV). One of the two structures proposed<sup>104</sup> for the aporphine alkaloid laurepukine has been eliminated by a synthesis<sup>105</sup> of 3 : 4-dimethoxy-6 : 7-methylenedioxyaporphine (XXXV).



XXXIII



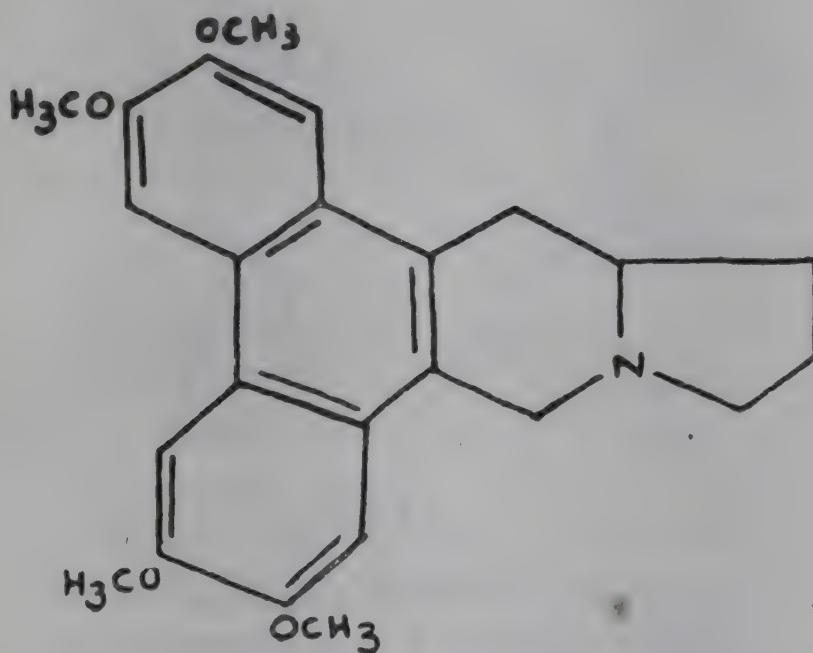
XXXIV



XXXV

Full details of the degradative studies carried out on tylophorine, the major alkaloid of *Tylophora asthmatica* have been published.<sup>106</sup> Vigorous oxidation of tylophorine methiodide gave only m-hemipinic acid. Treatment of the alkaloid with cyanogen bromide gave a bromocyanamide, which reacted readily with diethylamine indicating the presence of a benzylamino group. Facile conversion of the bromocyanamide to a hydroxycyanamide, which on hydrolysis regenerated tylophorine, proved the presence of 1 : 4- or 1 : 5-aminoalcohol system in the hydroxycyanamide. The reversion of tylophorine methine to tylophorine methohydroxide indicated that the alkaloid had the nitrogen common to two rings which are five or six-membered. Emde degradation of tylophorine methochloride to dihydrohomotylophorine and dehydrogenation of the latter yielded a pyrrole derivative, which regenerated the Emde base on hydrogenation. This definitely proved that one of the nitrogen rings was five membered. Hofmann degradation of the Emde base gave a basic compound which on oxidation followed by decarboxylation gave 2 : 3 : 6 : 7-tetramethoxy-9-methylphenanthrene, identified by synthesis. On these grounds tylophorine has been formulated

as 2:3:6:7-tetramethoxyphenanthro (9:10-6':7') indolizidin (XXXVI).



XXXVI

A synthesis of the parent ring system present in tylophorine has also been accomplished.

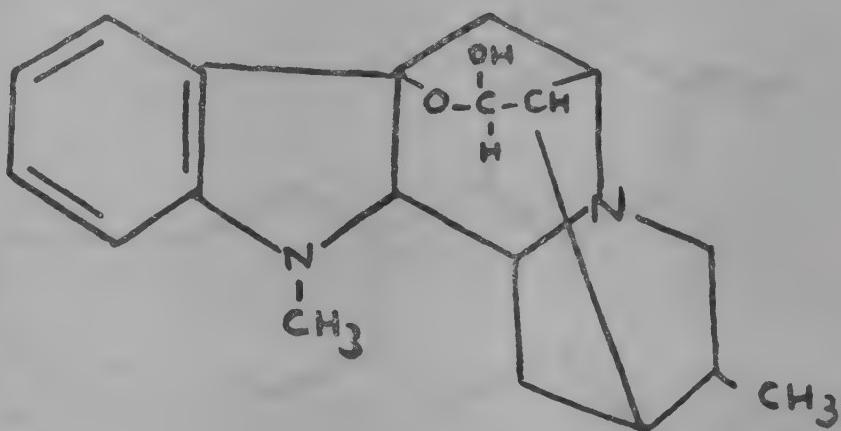
Umbellatine<sup>107</sup> has been shown<sup>108</sup> to be identical with a form of berberine m.p., 205° (decomp.) and neprotine with jatrorrhizine. Commercial Khet papra has been identified as *Fumaria parviflora* and Chauhan and Tewari's biflorine<sup>109</sup> has been shown to be protopine.<sup>110</sup> The principal alkaloid from the roots of *Cyclea burmanii* has been shown<sup>111</sup> to be identical with tetrandrine.<sup>112</sup>

A method for the isolation of serpentine from the roots of *Rauwolfia canascens* has been described.<sup>113</sup> Serpine and ajmaline have been isolated<sup>114</sup> from *Rauwolfia canascens* collected in W. Bengal. The 3:4:5-trimethoxycinnamoyl derivative of rauwolscine has been prepared.<sup>115</sup> It has strong hypotensive action but no reserpine-like tranquilising properties.

A structure (XXXVII) has been proposed<sup>116</sup> for rauwolfinine, mainly on biogenetic consideration.

On the basis of a study of molecular models and applying the principles of conformational analysis, the stereochemistry of

ajmaline, ajmalidine and rauwolfinine have been discussed.<sup>117</sup> Similarly, sarpagine has been assigned an absolute stereochemical formula.<sup>118</sup>



XXXVII

An alkaloid, macralstonie,  $C_{44}H_{54}O_5N_4$ , m.p., 270-272° (decomp.) has been isolated<sup>119</sup> from the trunk bark of *Alstonia macrophylla* in 0.06% yield. It does not contain a methylenedioxy group but contains one active hydrogen, one methoxyl and at least two C-methyl functions. N-methyl data suggest the presence of three or four such groupings. It is more hypotensive than rauwolscine ( $\alpha$ -yohimbine).

Using a new technique of 'divided columns' mixtures of alkaloids from *Rauvolfia micrantha*, *Lochnera rosea* and *Tabernamontana heyneana* have been separated.<sup>120</sup> It is claimed that far better separations are achieved by the new method than by the conventional method using undivided columns.

From *Inula royleana*, the isolation of three alkaloids and a yellow neutral substance have been reported.<sup>121</sup> A method for the industrial production of papaverine has been outlined.<sup>122</sup>

Attention has been drawn<sup>123</sup> to the possibility of using the steroidal alkaloids from *Solanum* species as starting materials for syntheses of sex hormones and of cortisone.

### Miscellaneous

The juice of *Passiflora edulis* has been shown to contain about 3.3% of citric and 0.18% of malic acids, total acid content

being 3.5%.<sup>124</sup> The phenolic content of spontaneum juices is found to be higher than that of cultivated canes.<sup>125</sup> The enzyme hydrolysis method of Hanes and the colorimetric method of Balch were applied to the estimation of starch in sugar-cane and the former method was found more accurate.<sup>126</sup> A systematic chemical examination of the root, middle and top portions of jute fibre has been carried out.<sup>127</sup> The variation in the holocellulose,  $\alpha$ -cellulose, xylan and polyuronide content is not significant. The root portion is more lignified than the top portion. Fat and wax contents are generally higher at the root end. The top portion of the plant is richer in nitrogen.

The pericarp of *Emblica officinalis* has been shown<sup>128</sup> to contain fatty matter (6%), gallic acid (5%), phyllemblic acid,  $C_{16}H_{28}O_{17}(COOH)_8$ , m.p., 222-224°, a new crystalline phenolic product emblicol,  $C_{26}H_{30}O_{19}(OCH_3)_6$ , m.p., 191-194°, ellagic acid and other products. Some non-crystalline bitter principles have been isolated from the seeds of *Caesalpinina bonducella*.<sup>129</sup>

The carotenoid pigments in *Passiflora edulis* (passion fruit) have been analysed<sup>130</sup> using the phase separation and chromatographic techniques. The following ranges of variation have been observed in the major groups: free xanthophylls, 10.3-21.5%; xanthophyll esters, 11.1-34.6%; and epiphasic non-saponifiables, mostly carotenes, 45.7-76.3% of the total carotenoid pigment content.

A simple, rapid and reliable method for the determination of carotene in the Indian lichen *Roccella monatguei* has been worked out.<sup>131</sup> Samples of lichen from Waltair uplands have been found to contain 1.5-2.0 mg. of carotene/g. of air-dried material.

Two new crystalline substances, m.p., 103-104° and 153-154° respectively have been isolated<sup>132</sup> from the root bark of *Mundelia suberosa*. The first has the molecular formula  $C_{18}H_{18}O_3$ , and contains one methoxyl group and an  $\alpha : \beta$ -unsaturated carbonyl system, probably as a lactone. The second has the molecular formula  $C_{16}H_{16}O_3$ , and possesses a bonded hydroxyl and a  $\alpha, \beta$ -unsaturated carbonyl group.

Aristolochic acid and an orange-yellow compound have been isolated<sup>133</sup> from the seeds of *Aristolochia bracteata*. The seed also contains an oil sp.gr. 0.908, sap. value, 195 and iodine value, 92.

Three benzenoid trimethyl ethers different from pristimerol dimethyl ether have been obtained<sup>134</sup> by methylation of pristimerin with dimethyl sulphate and potassium carbonate in acetone. On reduction with lithium aluminium hydride, pristimerin yields products with the quinonoid system intact but without the double bond originally present in conjugation with it. On ozonolysis, pristimerin yields a volatile product forming a 2:4-dinitrophenyl-hydrazone, m.p., 145-146°. On oxidation with sodium perborate, pristimerin yields a lactone monocarboxylic acid, a dicarboxylic acid and a dilactone.

Based on the analysis of over five hundred samples of South Indian black tea, the average composition of the tea has been found<sup>135</sup> to be: total ash, 6.02%; water-soluble, 3.48%; acid-soluble ash, 0.42%; total nitrogen, 4.58%; crude protein, 22.57%; caffeine, 3.3%; crude fibre, 11.75%; tannins, 13.13%; and water extractives, 40.13%.

A broad view of the problem of utilisation of Indian medicinal and allied plants and the work already carried out at the Regional Laboratory, Jammu, in this connection has been given by Chopra *et al.*<sup>136</sup>

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## VITAMINS

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During the year under review much progress has been made in the field of vitamin research. The symposium on "Biosynthetic and Metabolic Aspects of Vitamins," held at Mysore in October, 1958, under the auspices of the C.S.I.R., brought out a number of interesting papers, the abstracts of which have already appeared.

In the following pages, a review of the work done in India on the subject of vitamins during the year has been made.

### **Carotene and Vitamin A**

Krishnamurthy and Sankara Subramaniam<sup>1</sup> have studied the carotene content of the lichen *Roccella montagnei* and found the average content of carotene to be 1.5 to 2.0 mg./g. of the air-dried lichen.

The importance of carotene in cattle feeding and animal nutrition has been emphasised in an article by Banerjee.<sup>2</sup>

The stability of sesamolin in Vanaspati in the presence of vitamin A acetate has been investigated by Dane and Ananthanarayanan<sup>3</sup> who have found that vitamin A and sesamolin are stable at room temperature in the dark when contact with air is avoided, while samples with sesamolin alone deteriorate when stored similarly.

Pai<sup>4</sup> has studied the vitamin A content of several articles of foods in their pre-cooked and cooked stages. The loss of vitamin A in all preparations varied between 10 and 70%. In cereal preparations the range is from 10 to 38%, in vegetables from 34 to 40% and in dals from 57 to 70%.

Balasundaram *et al.*<sup>5,6</sup> have isolated anhydrovitamin A<sub>2</sub> and a new compound named rehydrovitamin A<sub>2</sub> (N) from the liver oil of the freshwater fish *Wallago attu* and studied their spectroscopic characteristics. It has been found that anhydro-

vitamin A<sub>2</sub> can be formed by treatment of vitamin A<sub>2</sub> alcohol with anhydrous ethanolic hydrogen chloride but not with p-toluene-sulphonic acid which confirmed the presence of an ethoxy group in anhydrovitamin A<sub>2</sub>. Anhydrovitamin A<sub>2</sub>, when fed to vitamin A-deficient rats, is transformed into a new compound named rehydrovitamin A<sub>2</sub> which is stored as an ester and alcohol in the liver. A tentative structure for rehydrovitamin A<sub>2</sub> has been proposed and its absorption spectra in different solvents have been given.

Cama and Sundaresan<sup>7</sup> have investigated the biopotency of crystalline *all-trans* retinene<sub>2</sub> (vitamin A<sub>2</sub> aldehyde) isolated from the freshwater fish liver oil of *Wallago attu* species and, using the U.S.P. growth test found it to be 47% compared to crystalline vitamin A acetate. Maleic anhydride adduct formation with vitamin A<sub>2</sub> alcohol, obtained by the lithium aluminium hydride reduction of retinene<sub>2</sub>, has shown that the crystalline retinene<sub>2</sub> on which the biopotency is determined is entirely in an *all-trans* form.

The reaction of maleic anhydride with unsaponifiable fractions of freshwater fish liver oils and vitamin A<sub>2</sub> concentrates has been studied by Cama *et al.*<sup>8</sup> with a view to investigate the isomeric forms of vitamin A<sub>2</sub>. It has been found that in high potency oils, the recovery of vitamin A<sub>2</sub>, ranged between 3 and 6% while in low potency oils, the recovery is between 9 and 12%. Similar studies on vitamin A<sub>2</sub> esters and vitamin A<sub>2</sub> alcohol obtained by lithium aluminium hydride reduction of retinene<sub>2</sub> showed low recovery of vitamin A<sub>2</sub>, thus indicating that vitamin A<sub>2</sub> existed mainly in an *all-trans* form.

Cama and Malik<sup>9</sup> have observed that supplementation of groundnut protein with penicillin, aureomycin, streptomycin and vitamin B<sub>12</sub> significantly reduced the liver reserves of vitamin A esters in the rat when preformed vitamin A is included in the diet.

The intracellular distribution of vitamin A esterase in chicken and rat livers has been studied by Krishnamurthy *et al.*<sup>10, 11</sup> It has been found that in the rat liver, vitamin A esterase and cholesterol esterase activities are confined to the microsomal fraction alone while in chicken liver, they are distributed between

the nuclear and microsomal fractions. Esterified cholesterol is distributed between the microsomal and supernatant fractions whereas free cholesterol is located in the microsomal fraction. All of the vitamin A ester and most of the alcohol were found in the supernatant fraction. In an attempt to study the mechanism of storage of vitamin A ester and vitamin A alcohol in the rat liver using the techniques of protein precipitation, protein denaturation etc., Krishnamurthy *et al.*<sup>12,13</sup> came to the conclusion that vitamin A ester and vitamin A alcohol are stored in the liver with two different lipo-proteins in the Kupffer and parenchymal cells, respectively. Krishnamurthy *et al.*<sup>14</sup> have further studied the absorption and storage of carotenoids and vitamin A in chicken and found lutein to be in the intestinal muscles, whereas all the other carotenoids are found to be present only in traces in the mucosal cells. Vitamin A ester appears mostly as an ester in the intestinal muscles and in the supernatant fractions of the mucosal-cell homogenate.

In a comparative study of esterase, vitamin A esterase and cholesterol esterase, Sastry *et al.*<sup>15</sup> have found that the three activities are not identical. Data on the properties of these enzymes are discussed.

Raju and Rajagopalan<sup>16</sup> have found that utilisation, absorption and storage of vitamin A and carotene are adversely affected when the vitamin is fed in heated oils to rats.

### Vitamin D

Dikshit *et al.*<sup>17</sup> in a study on the mode of action of vitamin D, have investigated the effect of starvation on the healing of rickets, changes in the citrogenase activity and citrate and total phosphorus in the epiphyseal cartilage. Starvation induces healing of the rachitic lesion, unaccompanied by increase in citrate or citrogenase activity in the epiphyseal cartilage. The total phosphorus content of the latter, however, increases significantly. The authors present a tentative hypothesis of a common pathway of action of starvation, citrate and vitamin D in the healing of rickets.

### Vitamin E

Nair and Magar<sup>18</sup> have observed that the administration of molybdenum to albino rats causes increased excretion of urinary phosphorus and creatine, accompanied by a decrease in creatinine excretion. Simultaneous doses of vitamin E reverse the effect of molybdenum and induce a decrease in excretion of molybdenum by an *in vivo* retention in the tissues.

Nazir and Magar<sup>19</sup> have investigated the phosphomolybdic acid-tocopherol reaction. Specificity experiments with free and esterified vitamin A<sub>1</sub>, calciferol, cholesterol, vitamin K<sub>1</sub>, β carotene, and experiments to determine the effects of fats, fatty acids and peroxides on the phosphomolybdic acid—tocopherol reaction showed that there exist comparatively very few substances which interfere with the reaction.

Srikantan and Krishnamurti<sup>20</sup> have reported the effect of tocopherol deficiency on tissue enzymes of the growing rat. Liver and brain succinic dehydrogenase activities have been found to be of the same order in both control and tocopherol-deficient rats, but the activity of the kidney increased by 300% in vitamin E deficiency. Xanthine dehydrogenase also increases in all the tissues in vitamin E deficiency.

Ramanujam and Ananthakrishnan<sup>21</sup> have reported on the tocopherol content of ghee. It varies from 20-45 µg./g. of fat and mixing with extraneous vegetable fat, it increases and this method has been used for detection of adulteration. They<sup>22</sup> have also studied the relative stability of tocopherol in ghee and other edible oils, during storage and heating and found the stability of tocopherol in ghee, hydrogenated groundnut oil and coconut oil to be more than that in groundnut and sesame oil. The same authors<sup>23</sup> have reported the influence of growth, season and method of drying on the tocopherol and carotene contents of a variety of grasses. The relative distribution of tocopherol and carotene in different parts of the leaf has also been investigated.

### Thiamine

Studies on metabolic effects of oxythiamine in *Neurospora* and the reversal of these effects by thiamine and acetate have

been reported by Sivasankar.<sup>24</sup> Growth inhibitory action of oxythiamine on *Neurospora crassa* is completely reversible by either thiamine or by acetate. Succinate and citrate also could reverse the inhibition to a large extent, while a number of compounds including acetaldehyde, palmitate and acetoacetate could not reverse toxicity of thiamine. It has also been observed that *in vivo* oxythiamine does not decrease the pyruvic decarboxylase activity of the small amount of mycelium, but *in vitro* addition of the antivitamin abolished the enzymatic activity. This inhibition is completely counteracted by thiamine and not by acetate.

### Riboflavin

Joshi and Sohonie<sup>25</sup> have reported that riboflavin in palm gur is present in a readily available form.

The effects of vitamins in various combinations, sugars, anti-vitamins, amino acids and purines on the growth of and flavino-genesis in *Eremothecium ashbyii* have been studied by Radha-krishnamurthy and Sarma.<sup>26</sup> The results obtained indicate that inositol and probably p-aminobenzoic acid or pteroylglutamic acid are concerned in the formation of rings B and C of riboflavin.

Giri and Appaji Rao<sup>27</sup> have reported on the enzyme system involved in the synthesis of flavine adenine dinucleotide (FAD) from flavin mononucleotide (FMN) and adenosine triphosphate (ATP) in plants. They have found that the several plant sources examined are capable of synthesising FAD enzymatically, the identity of which is established by spectrophotometric and chromatographic techniques. Further they have provided data on the characteristics of this enzyme from green gram. In another communication, Giri *et al.*<sup>28</sup> have reported the occurrence of enzymes synthesising FMN and FAD in milk. The enzymes from cow and human milk showed pH optima at 7.6 and temperature optima at 37°C.

### Nicotinic acid

Agarwal *et al.*<sup>29</sup> in a study on nicotinic acid-tryptophan metabolism in man, have examined twenty-four hour urinary excretions of nicotinic acid and amide and other intermediary

metabolites formed during the *in vivo* synthesis of nicotinic acid from tryptophan in adult men, before and after feeding of DL-tryptophan, on two consecutive days. There is an increase in the excretion of all the metabolites after the feeding. Kynurenin and 3-hydroxy anthranilic acid appeared in urine only after the feeding of tryptophan.

Further, Banerjee and Agarwal<sup>80</sup> have examined the urinary excretion of nicotinic acid and amide as well as various intermediary metabolites from tryptophan in various diseases, with a view to study the metabolism of nicotinic acid and its site of synthesis from tryptophan. Similar investigations on schizophrenic patients have also been made by the same authors.<sup>81</sup>

The metabolism of nicotinic acid and nicotinamide in *Neurospora crassa* has been studied by Sundaram *et al.*<sup>82, 83</sup> In this mould, free nicotinic acid is the chief end product of metabolism. The presence of a nicotinamide deamidating enzyme has been demonstrated in cell-free extracts of *N. crassa* and the deamidating activity has been found to be localised in the soluble portion of cytoplasm. Further, the same authors (Rajagopalan *et al.*,<sup>84</sup>) examined the livers and kidneys of several vertebrates for nicotinamide deamidase activity. Unlike majority of vertebrates, some avian species such as pigeon and the chick have been found to have very high deamidase activity. In pigeon, both liver and kidney have the activity while in the case of the chick only the kidney is able to deamidate nicotinamide.

Sengupta<sup>85</sup> has estimated microbiologically the nicotinic acid content of Indian food stuffs from acid hydrolysates using *L. arabinosus*. Colorimetric estimations have also been carried out. The microbiological method is found to be the best for estimation.

Shah *et al.*<sup>86</sup> have evolved a method for the determination of nicotinamide in B-complex and multivitamin preparations by the cyanogen bromide method. Silicotungstic acid is used to remove the interfering vitamin B<sub>1</sub> and to stabilise the colour developed for a reasonable time.

### Pyridoxine

Guha *et al.*<sup>87</sup> have demonstrated that rat and several other mammalian brain tissues contain an active enzyme system by

means of which 1-glutamine, without any prior hydrolysis, can directly participate in transamination-deamidation reaction in presence of several  $\alpha$ -ketoacids. The enzyme activity is entirely localised in mitochondrial fraction of rat brain. Effect of antipyridoxine compounds on the enzyme system indicated that the enzyme activity is dependent on the presence of vitamin B<sub>6</sub> or some of its active derivatives.

### Folic acid

Banerjee and Pain<sup>38</sup> have estimated urinary excretion of PGA and CF in a number of diseases before and after administration of PGA by both enteral and intravenous routes, with or without simultaneous administration of ascorbic acid. Patients suffering from liver cirrhosis, typhoid, renal hypertension, acute malaria, influenza and nutritional anaemia excreted diminished amounts of PGA and CF than normal subjects. After feeding PGA, these patients excreted lesser CF than normal persons. Administration of vitamin C along with PGA enhanced urinary excretions of CF in all diseased conditions, though this increase was less than that in normal subjects. The authors recommend the administration of PGA or CF in different diseased conditions.

Enzymic cleavage of folic acid by extracts from human blood cells has been studied by Arvindakshan and Bragance.<sup>39</sup> They investigated the liberation of the active form of enzyme and its specificity and found that the active form, liberated by heat treatment, has the same cofactor requirements as the ammonium sulphate fraction F<sub>2</sub>. Among the various ions tested for their effect on F<sub>2</sub>, Co<sup>++</sup> activated the system, while cyanide and azide inhibited it. None of the metals could replace Mn<sup>++</sup> as an essential cofactor. Vitamin C was found to be a potent inhibitor and its effect could be partially reversed by increasing the concentration of glutathione.

Bragance and Krishnamurthy<sup>40</sup> have shown that the conversion of folic acid into 2-amino-4-hydroxy-6-formyl pteridine and p-amino-benzoyl glutamic acid in the presence of glutathione and Mn<sup>++</sup> by human blood cell extracts is catalysed by a peroxidase. The enzymic conversion is inhibited by added catalase as well as by peroxidase inhibitors like cyanide and azide. Purified catalase

from blood and crystalline catalase are ineffective. Blood extracts containing the least amount of catalase are found to be the most potent in degrading folic acid.

Datta and Bhattacharya<sup>41</sup> have found that proteolysed liver preparation, on being treated with an excess of folic acid, produced a precipitate which is found to contain a basic protein body, folic acid and a polysaccharide. The basic polypeptide, which could also be separated from the proteolysed liver with a suitable ion exchange, is composed of eight amino acids, and combined with folic acid in the ratio of 1 : 26.

Shah *et al.*<sup>42</sup> have described a colorimetric method for the determination of folic acid in the presence of large amounts of vitamin C. The interference caused by vitamin C in the development of normal colour is removed by increasing the concentrations of sodium nitrite and ammonium sulphamate to 1% and 5%, respectively. They (Shah *et al.*<sup>43</sup>) also estimated folic acid in presence of iron salts by precipitating them with hydrated tribasic sodium phosphate but in the case of complex iron salts, the use of ethylene diaminetetraacetate (EDTA) has been found to be satisfactory.

Ghosh *et al.*<sup>44,45</sup> have synthesised three folic acid analogues which are derivatives of 2-amino-4-hydroxy pteridine-6-aldehyde and tested their effects on the growth of *S. faecalis*, *L. arabinosus* and *E. coli*. These three compounds are found to be inhibitory to the growth of *S. faecalis* and *E. coli*, but this inhibitory effect is feeble in the case of *L. arabinosus*. Moreover, all these three compounds are antagonists to folic acid and folinic acid in regard to growth of *S. faecalis*. Their inhibitory activity is reversible by excess of PGA and folinic acid but when folic acid is replaced by a mixture of adenine and thymine, the compounds lose their inhibitory action. Thymidine, reverses the inhibitory effects of these compounds on *L. arabinosus* while in *E. coli*, thymidine and serine individually or in a mixture can reverse the inhibitory effects.

### Vitamin B<sub>12</sub>

Mukherjee and Banerjee<sup>46</sup> have investigated the protein and carbohydrate metabolism as affected by the combined deficiency of vitamin B<sub>12</sub> and folic acid in rats. During the progress of the

combined deficiency, all the nitrogenous constituents, except creatine, such as non-protein N, urea N, uric acid, amino acid N and creatinine are excreted in large amounts. In the deficient animals, a high non-protein N in blood and a very low glycogen content in liver are noted. There is no change in protein contents of blood and liver in the deficient rats.

Balasundaram *et al.*<sup>47, 48, 49</sup> have studied the influence of supplementation of groundnut meal with antibiotics and vitamin B<sub>12</sub> on the nutritive value of the protein. Penicillin at a level of 0.02% of the diet significantly improves the nutritional quality of the proteins as manifested by increased growth rate, biological value and true digestibility coefficient. Aureomycin at 0.03% level and streptomycin at 0.02% level of the diet are effective only on the digestibility coefficient. Vitamin B<sub>12</sub> at 45 µg./kg. of diet improved the biological value of the protein but has no effect on growth in the paired-feeding test.

Mulgaonkar and Sreenivasan<sup>50</sup> have recorded observations on the binding of vitamin B<sub>12</sub> by rat serum protein fractions. Vitamin B<sub>12</sub> is found to exist chiefly bound to α<sub>2</sub>-globulin. On addition of 1.5 µg. of vitamin B<sub>12</sub> to 1 c.c. serum *in vitro* the bound vitamin increases from 0.4 µg. to 0.8 µg. per c.c. A close relationship is observed between the concentration of the bound vitamin in whole serum and in α<sub>2</sub>-globulin, and between the free vitamin concentration and the β-globulin bound vitamin. These authors suggest that vitamin B<sub>12</sub> on entering the blood is bound by α-globulin and transferred by β-globulin to its functional sites.

In another communication, Mulgaonkar and Sreenivasan<sup>51</sup> have reported on the alterations in rat serum proteins in single deficiencies of folic acid and vitamin B<sub>12</sub>. In vitamin B<sub>12</sub> deficiency there occurs a decrease of serum albumin and α<sub>1</sub>-globulin. In folic acid deficiency a significant decrease in β- and γ-globulins is noted. In vitamin B<sub>12</sub> deficiency, increase in β-globulin is accompanied by increase in relative concentration of free vitamin B<sub>12</sub> in serum. These authors have suggested that in the deficiency state a preferential synthesis of β-globulin occurs whereby available vitamin is mobilised.

Baker *et al.*<sup>52</sup> have studied the site of absorption of small amounts of vitamin B<sub>12</sub> from the gastrointestinal tract of the dog. Evidence is presented that the site of absorption is in the second half of the small intestine and that little or no absorption occurs in the stomach, jejunum or colon.

The distribution of alkaline and acid phosphatases, RNA and DNA in normal, vitamin B<sub>12</sub>- and folic acid-deficient rats has been recorded by Banerjee and David<sup>53</sup> using histo-chemical techniques. The alkaline phosphatase increases in adrenals, spleen, testes and thyroid, in vitamin B<sub>12</sub>- and folic acid-deficient animals while the acid phosphatase increases in liver and adrenal and disappears in the pituitary.

Sundaram and Sarma<sup>54</sup> have investigated the metabolic fate of common methyl acceptor, glycocyamine in the insect *Corcyra cephalonica St.* No creatine or creatinine is found in the concentrated larval excreta which indicated the failure of glycocyamine to get methylated in the system of *Corcyra* larva. Vitamin B<sub>12</sub> is also found to be entirely absent in the *Corcyra* larval tissues.

Baliga *et al.*<sup>55</sup> have studied the influence of vitamin B<sub>12</sub> on the biological value of autoclaved soyabean with different percentage levels of proteins in the diet. Their results show that with increasing concentration of protein in the diet, the biological value is considerably lowered with vitamin B<sub>12</sub> and aureomycin individually and together.

The nutritional requirements of an indigenous strain of streptomyces for vitamin B<sub>12</sub> production has been studied by Pinto and Ramasarma<sup>56</sup> using chemically defined media. Potassium, magnesium, calcium, zinc and cobalt are found to be essential for vitamin B<sub>12</sub> production. Vitamin B<sub>12</sub> synthesis requires organic nitrogen sources such as amino acids, of which aspartic acid is found to be essential. Vitamin B<sub>12</sub> production is improved by the addition of 0.25% agar and 150 mcg./ml. of orthophenylene diamine or benzimidazole.

Roy and Mitra<sup>57</sup> have discussed the metabolism of *Streptomyces griseus* and *Streptomyces olivaceus* in relation to vitamin B<sub>12</sub> synthesis. They have standardised the optimum conditions necessary for the maximum yield of the vitamin from indigenous

proteins of vegetable and animal origin. A new analogue of vitamin  $B_{12}$  has been obtained with *S. olivaceus* in a synthetic medium containing o-phenylene diamine. It has been noted that glycine and threonine are rapidly utilised from the medium during the growth phase of *S. griseus* and *S. olivaceus*.

Observations on the protective action of vitamin  $B_{12}$  against thyrotoxicosis in albino rats have been made by Sreenivasamurthy *et al.*<sup>58</sup> Their results have shown that vitamin  $B_{12}$  deficiency causes an impairment in the metabolism of depot fat in thyroxine-treated animals.

Bhattacharya and Bardhan<sup>59</sup> have carried out spectrophotometric assays of injection of cyanocobalamin in the presence of preservatives like phenol, p-chloro-m-cresol, cresol and benzyl alcohol using an aqueous solution of the preservative as the blank.

### Biotin

A comparative study of the effect of the micronutrients, biotin, manganese and calcium and jute extract on the growth and fruiting of chaetomium species is made by Basu and Bose.<sup>60</sup> Biotin is observed to exert maximum effect at a concentration of 10  $\mu\text{g}$  per 100 ml. of medium. All the three nutrients are required for the most favourable all round effect and this combination is found to be superior to 3 per cent jute extract from the point of view of mycelial weight and perithecial size but slightly inferior as regards total number of perithesia.

### Choline

The isolation and estimation of choline by a physicochemical method has been reported by Datta and Bose.<sup>61</sup> The method involves the use of cation exchange resin to isolate choline, freed from all amino acids and peptides. The base is finally reacted with ammonium reineckate for optical measurements.

### Vitamin C

The effect of glucose-cyclo-acetoacetate administration on the excretion of 17-ketosteroids in scorbutic guinea pigs and on the glutathione and cholesterol contents of the blood and adrenals has been studied by Bekhi *et al.*<sup>62</sup> The excretion of 17-ketosteroids

is not affected but the cholesterol content of the adrenals increases considerably. The depletion of ascorbic acid in tissues of deficient animals is less than that of the controls, the effect being more pronounced in the adrenals.

Nayudu and Nath<sup>63</sup> have examined the effect of acetoacetate on the adrenaline content of adrenals of normal and scorbutic guinea pigs. An increased secretion of adrenaline by repeated daily injections in both normal and scorbutic guinea pigs has been observed but the weights of adrenals are not affected.

Mohammed and Zaki<sup>64</sup> have determined the effect of Egyptian black snake toxin on ascorbic acid of adrenal cortex and on eosinophil level. A depletion of adrenal ascorbic acid with a marked eosinopenia is noted, when a sublethal dose of the toxin is administered, whereas by injection of a lethal dose of the toxin, no significant change in ascorbic acid content of adrenals is observed, though there is a significant eosinophilia.

Chatterjee *et al.*<sup>65-67</sup> have reported that rat and goat liver microsomes contain all the enzyme systems which can convert D-glucuronolactone into L-ascorbic acid in the presence of 0.005 M KCN. Further studies<sup>68</sup> revealed that the addition of soluble supernatant inhibited the cyanide mediated synthesis of ascorbic acid but the same soluble supernatant on heating not only lost the inhibitory power but on the other hand stimulated the ascorbic acid synthesis by rat liver microsomes in presence of lower cyanide concentration.

Roy and Guha<sup>69</sup> have found that liver tissues of birds such as chicken and pigeon which are known to synthesise their own requirements of ascorbic acid, failed to synthesise it *in vitro* from D-glucuronolactone in presence of cyanide. Similar studies on other animals showed that both amphibian and reptile kidney tissues but not their liver tissues could synthesise ascorbic acid.

The influence of vitamin C in the presence of Fe<sup>++</sup> and Mn<sup>++</sup> ions on the proteolytic activity of papain has been studied by Bahadur and Sinha<sup>70</sup> using casein as the substrate. The activation brought about by vitamin C, vitamin C+Fe<sup>++</sup> and vitamin C+Mn<sup>++</sup> is comparable to that brought about by the cyanide activated papain. Proteolytic activity increases with

increase in concentration of substrate and vitamin C but with increased concentrations of  $\text{Fe}^{++}$  and  $\text{Mn}^{++}$  in the form of vitamin C+ $\text{Fe}^{++}$  and vitamin C+ $\text{Mn}^{++}$  mixtures, the proteolytic power of papain does not increase.

Malakar and Guha<sup>71</sup> have reported on the isolation of bound ascorbic acid (ascorbigen) which they obtained in a relatively pure form.

Banerjee *et al.*<sup>72</sup> have determined the free and bound ascorbic acid contents of six varieties of common Indian pulses both before and after 48 hours and 96 hours of germination of the seedlings. Bound ascorbic acid is detected in all varieties of pulses and is found to increase significantly during the course of germination. In another report Banerjee and Singh<sup>73</sup> have examined the interrelationship between the insulin content of pancreas, the glutathione content of blood and tissues and the dehydroascorbic acid in the tissues of scorbutic guinea pigs. Mukherjee and Banerjee<sup>74</sup> have estimated ascorbic acid, dehydroascorbic acid and glutathione in blood in normal pregnant women and toxæmic patients. Blood levels of ascorbic acid and glutathione are significantly lower in the toxæmic patients. Dehydroascorbic acid is found to be absent in the blood of normal pregnant women but appeared in the blood of toxæmic patients.

Banerjee and Rohtagi<sup>75</sup> have analysed by paper electrophoresis the plasma proteins of normal and scorbutic guinea pigs, and recorded the changes in the concentrations of plasma proteins in scurvy.

Banerjee,<sup>76</sup> Banerjee and Singh<sup>77</sup> have discussed the metabolism of cholesterol in scurvy. The cholesterol content of adrenals, spleen and lungs decreases while that of testes and small intestine increases in scorbutic guinea pigs. Total body cholesterol significantly increases in scurvy and insulin treatment brings the cholesterol level of the intestine as well as the total body cholesterol to normal level in scorbutic guinea pigs. Insulin treatment also raises the acetylation of injected PABA to normal level.

Nath and Belkhode<sup>78-80</sup> have studied the biosynthesis of glucose-cyclo-acetoacetate and ascorbic acid in germinating *mung*

beans (*Phaseolus radiatus*). It has been found that glucose-cyclo-acetoacetate (GCA), a precursor of ascorbic acid in germinating legumes, is formed during germination from glucose and acetoacetate. While thiamine and pantothenic acid have been found to accelerate the biosynthesis of GCA, their antivitamins such as pyrithiamine and pantyltaurine depress such synthesis. This strengthens the possibility that GCA is an intermediate in the biosynthesis of ascorbic acid during germination of legumes.

Kuppuswamy *et al.*<sup>81</sup> have estimated the ascorbic acid levels of seeds of *Sesbania grandiflora Pers.* at different stages of germination. It has been found that ascorbic acid in Sesbanic seedlings is 40% more on the seed germinated without husk and seed coat than on whole seed and it is localised more in the radicle than in the cotyledon. They have also reported the effects of various additives like citric acid.

Brahmachari *et al.*<sup>82</sup> have analysed the edible portion of fruits of *Phyllanthus emblica* (Amla) for the ascorbic acid content and found it to be high and stable under different conditions such as sun-drying, powdering and storage in dark. In another study Brahmachari and Sharma<sup>83</sup> report on the ascorbic acid content of a variety of fruits and vegetables grown in desert areas of Rajasthan. The 2:4 dinitrophenylhydrazine condensation method has been adopted in the case of fresh fruits and vegetables. The stability of free ascorbic acid of an extract of edible portion of *Phyllanthus emblica* in 1% oxalic acid has been compared by Brahmachari and Gupta<sup>84</sup> with synthetic ascorbic acid solution under similar conditions. It has been found that the free ascorbic acid of the extract is far more stable than the synthetic ascorbic acid solution but the stability decreases considerably when the tannins are removed from the extract.

The vitamin C contents of citrus fruits in Darjeeling area have been analysed by Sarkar.<sup>85</sup> The vitamin C content of orange is found to increase with maturity and ripening. Sarkar *et al.*<sup>86</sup> have evaluated vitamin C in coloured preparations by reducing  $HgCl_2$  to  $Hg_2Cl_2$  by vitamin C and estimating mercurous chloride formed iodometrically.

## General

Pai<sup>87</sup> has studied the influence of cooking on the nutritional value of foods. Various food preparations have been chemically analysed for finding out the losses of thiamine, riboflavin and nicotinic acid contents.

Investigations by Pai and Patel<sup>88</sup> on the causative relationship between thiamine, riboflavin and nicotinic acid deficiencies and occurrence of toxæmias of pregnancy have shown that in this condition, the body is in a state of unsaturation with regard to thiamine and riboflavin but no state of unsaturation is revealed in the case of nicotinic acid.

Acharya *et al.*<sup>89</sup> have demonstrated the non-essentiality of vitamin B<sub>1</sub> for the biosynthesis of nicotinic acid from tryptophan through their investigations on three different biological systems, viz., the larva of *Corcyra cephalonica* St., germinating green gram seedling and resting mycelium. None of the thiamine antagonists could interfere with the biosynthesis of nicotinic acid from tryptophan.

Studies on the utilisation of riboflavin, nicotinic acid and biotin by some lactobacilli made by Nambudripad *et al.*<sup>90</sup> have shown that one of the strains indicated significant increase in riboflavin content of the medium during growth. In all other cases, there is loss of vitamins in the growth medium.

Bardhan *et al.*<sup>91</sup> have worked out a simple physico-chemical method for simultaneous and quantitative estimation of thiamine, pyridoxine and nicotinamide in the presence of riboflavin and pantothenol. Thiamine, pyridoxine and nicotinic acid have been estimated spectrophotometrically after removing strongly fluorescing riboflavin by passing the sample through a column of resin.

Sastray *et al.*<sup>92</sup> have observed that the lethal effects due to zinc toxicity in rice-moth larvae could be checked by vitamin B<sub>12</sub> and thiamine. Vitamin B<sub>12</sub> has been more effective in prolonging the survival of the larvae than promoting growth in this condition.

The influence of ingestion of curds in the diet on the intestinal synthesis of B-complex vitamins has been investigated by Baliga and Rajagopalan.<sup>93</sup> It has been reported that the

coliform bacteria increased in number in the intestinal tract. Also, there is a substantial increase in the amount of thiamine synthesised.

Raju and Rajagopalan<sup>94</sup> have provided information on the loss of vitamins in diets containing heated oils due to storage. Most of the B-complex vitamins are lost in different percentages and vitamin A has been found to be completely destroyed in 6-8 hours.

Sawant and Magar<sup>95</sup> have reported that the carotene retention values after 12 months storage at 37°C in four varieties of canned vegetables vary from 75 to 86.9% while the corresponding vitamin C retention values are 32.6 to 38.9%. The Fe content of all vegetables steadily increases at 37°C.

Nandi<sup>96</sup> has investigated the effect of darkness and ultraviolet irradiation on the free amino acid and B-vitamin contents of some leguminous seeds during germination. Thiamine, riboflavin and nicotinic acid increase greatly both in diffused light and darkness. Pantothenic acid remains practically the same except in one case. Folic acid is observed to decrease greatly with germination under diffused light. The changes in the free amino acid make up in darkness and diffused light have also been recorded.

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## SOILS & FERTILIZERS, 1957-1958

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### Soil Classification, Soil types

An excellent and fairly informative study of the genetic types of soils on the territory of India has been made by Academician Gerassimov<sup>1</sup> during the course of his recent visit to this country.

Menon and Mariakulandai<sup>2</sup> stated that the black soils of Madras are derived from granites and gneisses containing soda lime felspar and generally range from clay to heavy clay. Clay minerals chiefly present belong to the montmorillonite group. In a second paper<sup>2a</sup> they dealt with the red soils of Madras, including red earths, lateritic and laterite soils.

Soils of West Bengal have been classified under different categories and their characteristics studied by Chakravarti and Chakravarti.<sup>3</sup> The distribution of each kind of soil was shown in a soil map.

From profile and physico-chemical studies, Sinha<sup>4</sup> found the black soil of Padegaon to be of a typical 'regur' nature. Montmorillonite was uniformly present in the profile.

Soils of Rayaguda (Orissa) were classified as Kondolites with laterite peaks by Ramchander and Rao.<sup>5</sup>

Raychaudhuri and Biswas<sup>6</sup> suggested a procedure for the classification of grasslands in India on the basis of Storie Index and physical features of the soil, texture of surface soil, permeability, percentage of slope and degree of erosion.

The classification of soils into main soil types, based on profile studies, morphological characters and laboratory examination in the white sugar belts of Uttar Pradesh and the Punjab, viz., Rohana kalan (Muzaffarnagar), Beheri (Bareilly) and Phagwara (Punjab) Factory zones, was carried out by Singh and Chatterji,<sup>7</sup> Singh *et al.*<sup>8</sup> and Sharma *et al.*<sup>9</sup> From the viewpoint of cane growing, they discussed the suitability of the different types of soils.

Mehta *et al.*<sup>10</sup> devised a numerical land rating system for irrigability based on values of soil characteristics. Six profile characters were considered for rating and the method applied to soils of Chambal commanded area in Rajasthan.

Raychaudhuri<sup>11</sup> gave an account of the division of land according to its suitability for cultivation as was practised in ancient times.

### Soil formation

A pedo-chemical study of the soils of the Vindhyan plateau in U.P. was made by Agarwal *et al.*<sup>12</sup> It was pointed out that the process of soil formation was similar to one that gives rise to tropical and sub-tropical red loams.

Soil formation in alluvial lowland belonging to the lower Gangetic plains of eastern U. P., which gives rise to 'dhankar' soils (heavy textured soils in low lying tracts) and their cogeners, was studied by Gupta *et al.*<sup>13</sup> In this study several pedogenic characters were brought out and development of these soils discussed in relation to eluvial uplands; Murthy and Kumar<sup>14</sup> observed that in the soils of western U. P. which are not subject to the effect of floods, soil forming processes begin their work and bring about horizon differentiation and creation of soil profile. Climate has a profound influence. Illuviation of the profile is both intense and deep.

Tamhane and Lote<sup>15</sup> studied the soils of Simla Hills occurring at altitudes of 7000-8000 ft. The parent material, micaceous in nature—low grade chlorite schists—has markedly influenced the soil formation. The circulation of bases through plants checks intense unsaturation and breakdown of colloids. The vegetation also accounts for the stability of clay complex which maintains  $\text{SiO}_2 : \text{R}_2\text{O}_3$  ratio at a level of 2.2. The soils are more akin to Alpine humus soils than Brown Forest soils.

### Soil survey, mapping and land use

Out of 3.3 million acres surveyed in the Punjab by Dhawan *et al.*,<sup>16</sup> approximately 61 per cent was found fit for cultivation after introduction of irrigation, 30 per cent was in different stages of deterioration and 9 per cent excessively sandy.

Sharma and Rao<sup>17</sup> surveyed the apple growing soils of Himachal Pradesh on the basis of 2 mile grid ; hydrous mica was found throughout the profile.

Gupta<sup>18</sup> observed that the desert soils of Rajasthan contain phosphates in fair amounts. The aeolian sand is calcareous. Salt content is not in toxic doses. In stabilized sandy areas, leaching down of lime by rain has formed *kankar* pans in sub-soil.

The water-logged soils of Hamira (Punjab) sugar factory zone are characterized by high water table, soluble salt concentration and  $\text{CaCO}_3$  in the A horizon. The soils are heavy in texture as was observed by Sharma and Singh.<sup>19</sup>

Bhattacharya<sup>20</sup> carried out a land utilization survey in a laterite region in West Bengal. Land was classified and tentative programme of land use planning drawn up.

A rapid reconnaissance survey in an area of about 350 sq. miles (15 per cent to go under submergence), in the Koyana Project was taken up by Kibe and Puranik<sup>21</sup> with the object of assessing the patterns of erosion and subsequently with a view to recommend a system of land use planning.

Joshi,<sup>22</sup> after a thorough survey of the soils to come under the Tungabhadra project (gross commanded area 1,080,000 acres and area under irrigation 580,000 acres), prepared maps for proper land use planning.

### Soil erosion-runoff

Soil erosion study in laterite area in West Bengal, comprising of an area of 5000 acres of upper catchment area of Kaliaghai river, was made by Bhattacharjee.<sup>23</sup> Erosion is mainly due to water. 'Erosion ratio' was found to be affected by clay contents, colloid contents and pore space to a marked degree. Some remedial measures were also suggested. Narayanaswamy and Rege<sup>24</sup> critically examined the erosion problems in the Nilgiris in relation to soil, climate, rainfall, ground cover and farming practices and suggested suitable steps to tackle them. Working in Rajasthan, Mehta *et al.*<sup>25</sup> found erosion ratio of the soil to be correlated with clay ratio, dispersion ratio, pore size distribution, percolation and  $\text{CaCO}_3$  content and percolation rate with non-

capillary porosity, dispersion ratio and clay/moisture equivalent ratio. On the basis of these values soils have been classified as erodible and non-erodible.

Shabnam<sup>26</sup> brought forth the important problem of roadside erosion in Himachal Pradesh, which is tending to take alarming proportions. In the interest of road construction so far running to 1500 miles, the various soil conservation structural and vegetative cover methods are being overlooked and at places, worst type of roadside erosion in all its forms is witnessed. He suggested that an intensive programme of putting up soil stabilizing structure, proper water disposal and planting of the roadsides should be seriously taken in hand.

According to Barucha and Shankarnarayan,<sup>27</sup> depletion of vegetation by overgrazing has resulted in serious erosion of the clay portion of the soil in the Western Ghats. Overgrazing has reduced the organic matter content and cation exchange capacity of the soils. Mirchandani *et al.*<sup>28</sup> observed that runoff from properly grazed and overgrazed fallow was about the same in Hazaribagh area but soil loss from the latter was 4 times more. No clear relationship between runoff and soil loss was noticed. Suitable intercropping practices could decrease soil loss.

### **Reclamation and conservation**

Agarwal<sup>29</sup> reported the successful reclamation of an area in Kanpur, lying idle for many years due to salts and consequent deterioration, by treatment with powdered gypsum and sannhemp green manure. In another area *dhaincha* was used with gypsum. Shah *et al.*<sup>30</sup> examined about 800 sq. miles of saline alkali or non-saline alkali land in the Little Rann of Cutch. They reported as high as 15 per cent soluble salts and 60 per cent sodium saturation in the exchange complex, the pH varying between 7.4 and 8.5. Reclamation is possible as the area contains as high as 300 tons of gypsum per acre and plenty of bore water for initial removal of soluble salts. Mitra and Shankar<sup>31</sup> were able to carry out amelioration of alkali soils with chemical fertilizers and organic matter like weeds.

Investigations carried out under the guidance of Dhar<sup>32</sup> for

the last 4-5 years, showed that organic matter like straw, green manures, molasses, etc., help in reclaiming saline and alkaline soils, the process of reclamation speeding up when phosphate is added to organic matter. For alkali soils, gypsum with rice husk was found to be useful.

Prakash and Chowdhary<sup>33</sup> stated that the soils in the sand dunes of Rajasthan are fairly fertile and reclamation is possible due to the availability of moisture and nutrients in them.

The programme of soil conservation in the Damodar Valley dealing with afforestation, water disposal, consolidation, control of gullies and assessment of fertilizer needs was drawn up by Hull *et al.*<sup>34</sup> and graphically represented through maps and diagrams. A consolidated account of the work carried out for over 20 years at Sholapur on the effect of different soil and crop management practices on soil and water losses was put forth by Kibe.<sup>35</sup>

### Soil physics

Rao and Ramacharlu<sup>36</sup> observed that the dry sieve analysis curves of Delhi soil heated to 60-1000°C were the same as for unheated soil. The wet sieve analysis, however, differed from each other. The stability of the aggregates towards water increased steadily with temperature upto 360°C at which they became quite stable. Ramacharlu<sup>37</sup> showed that the permeability of different soils bear a close relation to particle size distribution in the wet state than in the dry state. Soil aggregates disintegrate in contact with water, sand suffers the least and disintegration in saline and alkaline soils is high.

Berseem (*Trifolium alexandrinum*) grown with phosphate in Delhi soil not only resulted in higher fodder yields but also in higher percentage of water stable aggregates (size 1-5 mm), according to Chaudhury.<sup>38</sup>

Khan *et al.*<sup>39</sup> found that mother as well as water stable aggregates were maximum in Delhi soil receiving 9 ploughings and with increase in number of cultivations more soil moisture was conserved.

### Soil-water

Kibe<sup>40</sup> gave the important findings made at Sholapur since

1933 on moisture retention and preventing soil erosion in scarcity zones.

Ramacharlu<sup>41</sup> observed a direct relationship between the rate of evaporation of water from certain Indian soils and the order in which the particle size distribution varies in them.

The variation of soil moisture at different depths in a red soil according to season and rainfall was reported by Gopalakrishnan and Balasubramanian.<sup>42</sup> Chandnani *et al.*<sup>43</sup> found a fairly close agreement between tensiometer readings and moisture percentage in sandy loam soils and the instrument could serve as a good guide for irrigating wheat and potato. Elongation of sugarcane was found to be affected by a fall in soil moisture in a study by Mallik and Venkataraman.<sup>44</sup> Naidu and Krishna Rao<sup>45</sup> attributed the reason for dry root rot in citrus in Andhra to the existence of hard pan in sub-soils in the affected plots leading to unfavourable soil-air-moisture relationship.

Basak<sup>46</sup> noted that at about 30 inches level of rainfall, 12.56, 11.86 and 10.46 in. of water were lost by transpiration, evaporation and percolation respectively during the growing period of paddy in West Bengal. The water requirements of paddy from transplanting to harvest and seed sowing to harvest were calculated at 30 and 45.5 acre inches respectively.

Desai *et al.*<sup>47</sup> reported that in poorly drained soils of Hyderabad, paddy suffers from sulphide toxicity with sulphatic fertilizers but it can be avoided by providing moderate drainage.

The quality of canal irrigation water in U.P. has been described by Agarwal *et al.*<sup>48</sup> as quite safe despite their use for the last 40 years and in spite of the fact that there has been spread and intensification of soil alkali, that is mainly due to the nearness of water table and impedance in downward movement.

According to Desai *et al.*<sup>49</sup> paddy could well tolerate a concentration of 0.2 per cent salt ( $\text{NaCl}$ ) in irrigation water, a concentration of 1 per cent proving fatal. Mehta and Desai<sup>50</sup> observed that chlorides of sodium and calcium present upto a concentration of 2.5 per cent in the soil solution at field capacity delayed the germination of most crops. Higher concentration severely affected the crop growth.

## Soil conditioners

In laboratory studies with Krilium and Poly-ack, improvement in the percolation rate in a few selected Bombay soils was observed by Gokhale *et al.*<sup>51</sup> There was an increase in water stable aggregates and improved water relation in heavy soils, but availability of potash and phosphate was not affected. Nath and Nagar<sup>52</sup> found that sticky point moisture of soils and clays was increased when treated with Krilium, SMA (co-polymer of styrene and maleic acid) and HPAN (hydrolysed polyacrylonitrile). Krilium, in most cases, proved to be better than the other two.

Krilium applied at 1 lb. per 100 sq. ft. was found by Kamat<sup>53</sup> to be completely effective in arresting erosion in deep black clay soil and about 40 per cent in the case of alkali soil.

Tejwani and Venkataraman,<sup>54</sup> however, did not find Krilium useful in improving the soil conditions in flue cured tobacco nurseries.

## Physical chemistry

A study of exchange equilibrium reactions involving measurements of cation activities in a mixture was carried out by Bose.<sup>55</sup> The activity of individual ions in a binary mixture was evaluated in order to study the reaction with the clay system:



A 'hysteresis' effect was present even if two similar valency ions were concerned. Chatterjee<sup>56</sup> demonstrated the differences in the exchangeability of a particular cation at different degrees of saturation by allowing the hetero-ionic systems,  $\text{NH}_4 = \text{Ba-clay}$  and  $\text{NH}_4 = \text{Na-clay}$  to equilibrate with H-resin in cellulose bags. Bose<sup>57</sup> demonstrated the suitability of membrane electrodes having different mobility ratios for the same cation pair for the determination of individual ion activities in a mixture.

In soils of varying texture and organic matter content, the relative rates of leaching of the 4 cations were, according to Banerjee<sup>58</sup> fastest for Na followed by K, Mg and Ca.

Exchange behaviour of Cu<sup>++</sup>, Mn<sup>++</sup> and Zn<sup>++</sup> ions was studied by Basu<sup>59</sup> by using aqueous suspensions of H-clay. The whole

quantity of adsorbed metal ions could not be released by dilute acid leaching, thereby suggesting a portion being rendered non-exchangeable or fixed by the clay. The metal ions exchanged hydrogen ions generally in the sequence Cu-Mn-Zn and were themselves released from the clay salts by other ions in the reverse order.

Chatterjee<sup>60</sup> carried out anion exchange measurements with particular reference to phosphate ions using electrodialysed clay fraction of a sample of kaolinite. Anion exchange resins were used for exchange measurements. Hydroxyl, citrate and oxalate but not fluoride ions of the anionic resin exchanged themselves for phosphate in varying extents.

Chakravarti<sup>61</sup> investigated if the extraction of nutrients could be carried out through the interposition of a septum or a membrane which is permeable to nutrient ions but not to the clay particles. Ca, Mg and K could be extracted by acetate buffer (pH 4.8) through cellophane membrane although in lesser amounts than in direct contact with soil.

Puri *et al.*<sup>62</sup> converted charcoals from sugarcane, coconut shells and cotton stalks into Na, K, Ba, Ca and NH<sub>4</sub> charcoals and titrated against H-soils and H and Ca-charcoals were titrated against Na-soils. Charcoals have a much higher cation exchange capacity than soils. Acid soils could be successfully titrated against alkali charcoals. K, Ca and NH<sub>4</sub> ions could easily be transferred from charcoal surface to soil surface resulting in lowering of soil acidity and enhanced supply of nutrients.

### Clay minerals; Clay

Investigations on the physical and chemical properties of the various types of clay available at Neyveli (S. India) were carried out by Ratnam and Veeraraghavan.<sup>63</sup> Adhikari<sup>64,65</sup> described the physico-chemical properties of some West Bengal clays. The electrometric curves, X-ray and differential thermal analysis (D.T.A.) diagrams were particularly improved in their features on removal of free oxides from the clays. His study<sup>66</sup> of D.T.A. showed the individual characteristics of clay minerals in mixtures and was, therefore, useful in identifying the nature of clays in simple mixtures.

A study of the electro-chemical, viscometric and swelling characteristics of pure clay minerals and their mixtures was reported by Chakravarti.<sup>67</sup> Adhikari<sup>68</sup> studied the symmetry values and adsorption isotherms of NH<sub>4</sub>-clays when exchanged with KCl and BaCl<sub>2</sub>. It seemed possible to identify the soil clays from the study of exchange isotherms without removing free inorganic oxides.

Mitra and Prakash<sup>69</sup> observed that montmorillonite shows the greatest adsorption of K at all pH values. According to Chatterjee and Ray,<sup>70</sup> appreciable amount of K was fixed when montmorillonite was repeatedly leached with a solution of KCl even at room temperature. Fusion analysis of the original and treated H-montmorillonite showed that the one prepared from the K-clay (by treatment with KCl) contained 3.01 per cent K while the original montmorillonite contained 0.5 per cent.

Mitra and Prakash<sup>71</sup> studied the chemical absorption of P from dilute solution of monocalcium phosphate by vermiculite, montmorillonite, biotite and halloysite. A study of the neutralisation curves of pure clay minerals and their mixtures in presence of a few quaternary ammonium and pyridinium compounds was conducted by Chakravarti.<sup>72</sup>

According to Rama Moorthy *et al.*<sup>73</sup> the presence of active SiO<sub>2</sub> tetrahedra on the surface of the clay is responsible for the formation of fertile group of clay-humus complexes. Formation of fertile group is favoured by the presence of montmorillonite and of infertile one by kaolinite.

### Composition of soils

Menon and Sankaranarayanan<sup>74</sup> studying the general characteristics of the black soils of Tinnes tract (S. India) stated that they are heavy clays, the finer fractions forming  $\frac{2}{3}$  to  $\frac{3}{4}$  of the soil. They have a high base exchange capacity, over 50 m.e. per cent.

At Pampore (in Kashmir) some soils grow saffron very well and some do not. Dutta Biswas *et al.*<sup>75</sup> made a study of the soil differences which may be responsible for such a behaviour.

From a study of organic matter and nitrogen contents of Indian soils of different altitudes Raychaudhuri and Sen<sup>76</sup>

observed that they tend to increase with altitude and rainfall. Nitrogen in forest soil decreases on cultivation with crops but a cultivated soil gains in nitrogen when left fallow or under pasture.

According to Nagar and Bhattacharya,<sup>77,78</sup> the cation exchange capacity of soils, however, was not dependent on the leguminous and non-leguminous nature of the humified plants and the nature of soil, physico-chemical properties of clay-humus complex, etc., may predominate.

Deshpande *et al.*<sup>79</sup> observed that the available phosphate in soils increased by introducing groundnut in rotation with cotton than growing cotton continuously or with *jowar* in rotation.

From a study of the ammoniacal nitrogen content of air dry surface soils from several parts of India, Sen *et al.*<sup>80</sup> found that it significantly increased with increase in moisture and decreased with increase in soil pH.

Biswas and Das<sup>81</sup> identified and estimated the free amino acids in soils with the help of paper chromatography. The overall concentration of free amino acids appeared to be higher in phosphate fertilized soil, particularly when *berseem* was in the rotation, than when no manure was used.

Black cotton soils of Beliary area, originating from granitic parent rock, were found to contain very low amounts of phosphate by Krishnamoorthy.<sup>82</sup> Khanna *et al.*<sup>83</sup> observed that phosphate fixation in heavy clay soil was higher than in light calcareous soil. The fixation tended to increase with time in heavy clay soil. Available phosphate in Ganga lowland soil was found to be more by Gupta<sup>84</sup> during the growing period of paddy than during non-growing period. P-availability in soil was highest at puddling and went on decreasing reaching minimum at the flowering stage.

The potash fixing capacities under continuously moist conditions of the Ca-saturated clay minerals showed characteristic differences according to the nature of the clay minerals (Mitra *et al.*<sup>85</sup>). Variations in red and alluvial soils could be explained on the basis that they contain varying but significant amounts of illite and kaolinite of free oxides which seem to have extremes of this capacity. According to Misra<sup>86</sup> when a soluble potash salt was added to the soil, a portion of the adsorbed K was transformed

into non-exchangeable form, the fixation increasing with the pH of the solution.

Raychaudhuri and Sinha<sup>87</sup> made a study of the saline and alkaline soils of Delhi State.

A statistical proof of seasonal variation of pH of the highly acidic loamy sand tea soils of Assam was furnished by Gokhale and Sen Gupta.<sup>88</sup>

Natarajan *et al.*<sup>89</sup> could detect, at intervals, very small traces of hydrogen sulphide liberated from submerged paddy soils, this offering no serious problem in paddy cultivation in Madras.

### Micronutrients

Water soluble boron contents in representative soils of Gujarat were determined by Gandhi and Mehta.<sup>90</sup> Satyanarayan<sup>91</sup> conducted a similar study with desert soils, where he found a high content of water soluble boron (2.6-12.2 p.p.m.). Gandhi and Mehta<sup>92</sup> observed that in the fine sandy loam soil of Bombay, boron leaches down to lower layers in the absence of any crop.

Khanna *et al.*<sup>93</sup> discussed the distribution of manganese in Sone alluvium soil division of South Bihar. No apparent relationship between the distribution of free  $\text{CaCO}_3$  or variation with soil pH with the depth distribution of Mn was observed.

A study of the micro-element distribution in I.A.R.I. (New Delhi) farm profile by Dutta Biswas and Dakshinamurti<sup>94</sup> showing abundance values of most of the elements, except copper, indicated that the soils originate from shales or like materials. Trace element status of cotton soils of Gujarat was reported by Satyanarayan.<sup>95</sup> Black cotton soils derived from igneous rocks showed higher total levels of trace elements than the sandy loams in North Gujarat.

The uptake of molybdenum, cobalt and zinc by a few forage plants of Western India was investigated by Iyer and Satyanarayan.<sup>96,97</sup> Verghese *et al.*<sup>98</sup> found only traces of strontium in coconut leaves and soils in Kerala.

Ghosh<sup>99</sup> examining town compost from different places in Bihar, reported that their percentage manganese contents were

between 0.035 and 0.072. No association could be found between total Mn, organic matter and phosphoric acid.

According to Joshi and Joshi,<sup>100</sup> agricultural soils in Maharashtra and Konkan regions of Bombay are deficient in copper to such an extent that it has a limiting factor in growth of crops. They obtained 25-80 per cent increase in crop yields (paddy and wheat) by supplying only small amounts of copper to such soils.

Preliminary studies on 'Citrus Die-back' in Coorg by Dikshit<sup>101</sup> showed that sprays with Zn and Cu sulphates tended to decrease significantly the intensity of chlorosis.

Manganese toxicity in rice was observed by Rao and Rao<sup>102</sup> in black soils of Nizamsagar and Tungabhadra area. The element is present in excessive quantity (0.7 per cent) as dark nodules and the plants develop necrosis of leaf tissue which is further intensified under ill-drained conditions.

In an attempt to find out spectral constituents especially trace elements, Kumar<sup>102a, 102b</sup> noted the presence of Mn, Mo and Co and apparent absence of Cu, B and Zn in all soil types of West Bengal.

### **Soil microbiology**

A new type of nitrogen fixing organism, different in features from Azotobacter and Pseudomonas and having high nitrogen fixing capacity was isolated by Roy and Mukherjee<sup>103</sup> from jute soils in W. Bengal. A new species of Azotobacter producing heavy slime and acid was isolated by Roy.<sup>104</sup>

According to Bhaskaran and Venkataraman,<sup>105</sup> a blue green alga, *Nostoc punctiforme*, which may fix atmospheric nitrogen, was found to occur with the rhizobial bacteria inside the nodules of berseem.

Sinha<sup>106</sup> studied the efficiency of Azotobacter as N-fixer and observed that its efficiency dies out with time and also that the bacteria are killed when exposed to sunlight. Iswaran and Sen<sup>107</sup> observed that nitrogen fixing capacity was adversely affected on prolonged exposure to 45°C, although when exposed to large diurnal change of North India temperature complete inhibition did not occur. They also showed<sup>108</sup> that sodium chloride affects N-fixation adversely, and its rate of decrease with increase in

NaCl was less for organisms isolated from soils of higher salinity than for those from less saline soils. Iswaran<sup>109</sup> recorded a higher amount of N-fixation by *Azotobacter chroococcum* in culture solution with humus obtained from legumes than from cereals.

That nodules from pea, treated with *mahua* cake, were small and distributed all over whereas with urea and ammonium nitrate they were larger was observed by Misra and Sen.<sup>110</sup> Rewari *et al.*<sup>111</sup> obtained definite evidence of excretion of nitrogenous substances from the roots of legumes to the soil, the amount varying between 8 to 21 per cent of the total N fixed by the legume. Superphosphate had an accelerating effect. Rewari and Sen<sup>112</sup> after isolating two pure strains of rhizobium, characterized by their acidic and alkaline nature, from single nodules of *guar* (*Cyamopsis psoraloides*) studied their N-fixing capacities.

Sen<sup>113</sup> reported that the legume *arhar* (*Cajanus cajan*) did not derive much benefit from root nodule organisms like other legumes. The crop was found to be of soil exhausting type and benefited from N and P fertilization.

Mineralization of nitrogen was slow in some typical soils of Bihar under aerobic incubation for first 6 weeks and then it rose sharply. Evolution of CO<sub>2</sub> correlated significantly with nitrogen mineralization and organic carbon content (Sinha *et al.*<sup>114</sup>). Bajaj and Subbiah<sup>115</sup> found leaching having a pronounced effect on nitrification, the amount of total nitrate produced by continuous incubation was 50-75 per cent of the total nitrate obtained by fortnightly leaching.

Sen and Rewari<sup>116</sup> stated that soil nitrogen decreases with increase in temperature and increases with increase in barometric pressure. Barucha and Wagle<sup>117</sup> observed that nitrifying capacity of soil was considerably lowered in storing in bottles for 24, 48 and 68 days.

N-fixation studies by Mehta and Sen<sup>118</sup> showed that there was increase in fixation by the mixed microbiological population with soluble phosphate indicating wide deficiency of phosphate in Indian soils. Sen and Paul<sup>119</sup> found that in cultures containing ammonium sulphate as source of nitrogen, *B. subtilis*, *B. mycoides*, *B. megatherium* and *B. mesentericus* released soluble P from cal-

cium and iron phosphates, Ca-glycerophosphate and lecithin.

### **Soil-macro and meso fauna**

Gokhale *et al.*<sup>120</sup> reported that in tea soils of Assam, termite activity results in a pronounced lowering of the acidity status of the soil. Termite affected soil is richer in potash and calcium but poorer in nitrogen.

Agarwal *et al.*<sup>121</sup> observed that certain species of earthworms in some Himachal Pradesh soils affect the soil structure adversely. The castings dry as cement hard clods and within a few years the fertile soil becomes cloddy, structureless and unproductive. Microscopic study showed that they cast some waxy fluid, which makes a gelatinous membrane around the castings and this is ascribed as a probable reason for clod formation.

### **Technique and analysis**

Working with alluvial soils of U.P., Khanna *et al.*<sup>122</sup> observed that the latest Bouyoucos hydrometer method for mechanical analysis gave fairly accurate values, comparable to those obtained in the International Pipette method, in almost all grades of soil, for fractions lower than 0.02 mm.

'Stability index' and 'Probable permeability' in soils, measured by Alderfer and Merckle's rapid method, were found to be positively correlated with pore space, apparent density and dispersion co-efficient and were thus useful in evaluating structural stability and permeability of soils (Nayar<sup>123</sup>). Seth and Yadav<sup>124</sup> suggested a simple field-cum laboratory method of determining soil permeability.

Seth *et al.*<sup>125</sup> developed a rapid colorimetric method with a wide range of clear colour changes for soils with high pH values. Khanna *et al.*<sup>126</sup> observed that pH values of non-calcareous soils of low soluble salt content, measured colorimetrically with suitable indicators, agreed well with electrometric measurements.

Perkin's method for rapid determination of cation exchange capacity of soil by saturation with Ca-Mg acetate and titration with EDTA, was examined by Iyer and Satyanarayan<sup>127</sup> for soils of different texture and they suggested certain modifications.

Ferric oxide in the HCl extract of soils was accurately estimated photometrically by Vanadate method by Rao.<sup>128</sup> Recovery of added iron in pure sand was near about 100 per cent. Kamalam and Mariakulandai<sup>129</sup> estimated accurately iron in soils by circular paper chromatography.

2-acetyl-pyridoxin, which gives a stable red colour with iron in alkaline medium (pH 10) and a stable yellowish green colour with copper salts, was reported by Tripathi and Banerjea<sup>130</sup> to be of good use as a colorimetric reagent for these elements.

Ray and Sarma<sup>131</sup> developed a colorimetric method for estimating cobalt by using alcoholic solution of dimethyl glyoxime with aqueous sodium azide at pH range 3.5-7.5. Mukherjee<sup>132</sup> suggested certain modifications of the colorimetric methods for estimation of Mn and Mo in acid extracts.

A study on the fertility status of Delhi soil by biological method using Jenny's technique, was carried out by Seth.<sup>133</sup> Lettuce was used as test crop and the results were found to be quite reproducible.

### Plant nutrition

Acharya and Jadav<sup>134</sup> and Acharya *et al.*<sup>135</sup> investigating on the chemical composition of wheat plant as a guide to its manurial requirements, observed that chemical analysis of the plant tissues and whole plant, carried out at different stages of growth, could indicate the nutritional status of the crop and thus help in assessing its manurial needs. Nayar<sup>136</sup> stated that the ash, silica and phosphate of wheat straw could be used as an index of soil productivity. Patel and Shah<sup>137</sup> reported that crude protein and phosphate contents in wheat fodder decreased with maturity, whereas crude fibre and calcium contents showed an increase. Sahasrabudhe *et al.*<sup>138</sup> observed the significant effect of nitrogenous manures in increasing nitrogen content in wheat grain.

Mariakulandai and Soundararajan<sup>139</sup> found that application of phosphate resulted in increasing the total and phytin phosphorus in *cholam* grains in Coimbatore Permanent Manurial experiments.

Sircar and Datta<sup>140</sup> observed that potash deficiency in paddy resulted in yellowish green colour of foliage, rapid die-back of leaves and death of tillers. The same degree of K deficiency was much more harmful when  $\text{NH}_4\text{NO}_3$  was the nitrogen source than when  $\text{NaNO}_3$  was used. Nitrogen nutrition of rice plants was studied by Tanaka *et al.*<sup>141</sup> and optimum level of nitrogen application was suggested.

An inverse yield-nitrogen content relationship in a number of sugarcane varieties was observed by Singh and Singh<sup>142</sup> and the yield potential was greatly influenced by their nitrogen content.

Satyanarayan<sup>143</sup> reported that *Heteropogon contortus*, a dominant grass of W. India, grows well on soils having a pH range of 5.75-6.10 and because of its narrow amplitude as regards soil reaction can be regarded as an indicator of medium acidity of soil.

### Organic manure; green manuring

Shirke and Narayana<sup>144</sup> carried out the analysis of a large number of town compost samples prepared according to Bangalore method in the Bombay State. The 6-8 month old compost from night soil and sweepings analysed to contain 0.9-1.11 and 1.5-2.0 per cent of N and  $\text{P}_2\text{O}_5$  respectively and were better than farm-yard manure. Bhaskaran *et al.*<sup>145</sup> showed that in the Bangalore method of composting human excreta and town refuse, all the pathogens and helminthic ova were completely destroyed in 3 months time. A well digested manure analysed to about 1 per cent nitrogen with a C/N ratio of 6-8.

Samples of leaf soil composts used in chincona nurseries were examined by Mukherjee and Chatterjee.<sup>146</sup> Leaf soils collected at higher elevation had higher nitrogen and organic matter and lower pH. P was generally low in all cases.

Rao *et al.*<sup>147</sup> discussed the utility of distillery slops in making composts with sugarcane trash.

Raju *et al.*<sup>148</sup> reported the results of agronomic experiments from different parts of S. India wherein the effect of night soil or town refuse compost on crop growth was found to be relatively better than FYM. Residual values of compost and FYM were

observed in most cases. Raju and Subramanyan<sup>149</sup> also discussed the method, scope and potentialities of the preparation of farm waste and night soil composts.

In an exhaustive article, Dhar<sup>150</sup> showed the importance of organic matter, preferably with phosphate in having a profound influence in increasing land fertility of the country.

FYM, compost and cotton waste manure, not only increased the yield of cotton but showed enough residual effect on *jowar* under rainfed cropping in black cotton soils, according to the findings made by Singh.<sup>151</sup>

Basak *et al.*<sup>152</sup> observed that compost gave significantly higher yields of paddy in all the 23 centres in West Bengal and for water logged paddy it was as efficient as ammonium sulphate.

Defatting (by solvent extraction) enhanced the manurial value of groundnut cake as tried on paddy by Desai and Rao.<sup>153</sup>

Desai *et al.*<sup>154</sup> found that tamarind 'chunie' (outer coat of seed—a by product of starch industry) contains 1.3 per cent nitrogen but has a poor manurial value for paddy as compared to groundnut cake. The seed, although inferior to cake, was found to be better than the 'chunie'.

Acharya<sup>155</sup> published a bulletin on preparation of fuel gas and manure by anaerobic fermentation of organic materials. An account of certain laboratory investigations carried out to examine the main factors which influence the quantity and quality of the combustible gas produced by anaerobic fermentation of organic materials is given followed by the description of some simple models of gas plants to suit village homes and farms and also experimental data relating to the working of the above models.

Analysis of different types of tree leaves, a good many of which can be used for green manuring purposes in view of their N-content and succulent nature, were reported by Nair *et al.*,<sup>156</sup> George and Kohli<sup>157</sup> and Patel and Patel.<sup>158</sup> According to Nair *et al.*,<sup>156</sup> green manure and season do not interact and nitrogen contents are more or less uniform throughout the years.

Nair *et al.*<sup>159</sup> profitably raised *Sesbania speciosa* for green manuring in laterite soils, preferably with lime, FYM or phosphate. Over 17000 lb. of green matter per acre were obtained by

Rajagopalan and Pawar<sup>160</sup> when sannhemp and *dhaincha* failed to grow well during the April—June period for summer rice.

Clusterbean (*Cyamopsis tetragonoloba*) was found to be a very useful green manure by Veeraswamy and Kunjamma.<sup>161</sup> In 60 days, it not only produced about 20,000 lb. of easily decomposable green matter, but also, in addition, 592 lb. of tender pods per acre, saleable as vegetable.

Khan and Mathur<sup>162</sup> observed that the percentage of nitrogen in sannhemp was highest in 4 week old plants but the amount of nitrogen added to the soil was more in 10 week old plants. However, the best results on wheat yield were obtained with 8 week old plants.

Desai and Rao<sup>163</sup> observed that green manure applied to ill-drained soils depressed the yields of paddy very appreciably.

Patil<sup>164</sup> reported that green manuring increased the yield of wheat by 54 per cent in Nasik district and it was better than 30 cartloads of FYM per acre.

Anant Rao *et al.*,<sup>165</sup> Khan and Mathur<sup>162</sup> and Rao<sup>166</sup> were able to get a substantial increase in the green matter output of sannhemp and *dhaincha* green manures and also high yields of wheat and paddy by adding phosphate. Desai *et al.*,<sup>167</sup> however, did not observe any beneficial effect of phosphate manuring of green manure in a soil rich in available phosphate. Sinha<sup>168</sup> reported that phosphate brought out an improvement in nitrogen availability in soil when used with green manure.

According to Chandnani,<sup>169</sup> sannhemp and clusterbean could add 90 and 70 lb. N per acre respectively in Delhi soil and the sowing of green manure is economical and compensates for the summer crop loss.

### Nitrogenous fertilizers

Chandnani and Kavitkar<sup>170</sup> stated from response curves that for wheat in Delhi soil 40 lb. N per acre is the optimum dose. Chandnani *et al.*<sup>171</sup> calculated that between 40 and 100 lb. N dose per acre, the loss in yield due to lodging varied between 25—50 per cent. An interesting observation that phosphate in presence of nitrogen induces lodging was made. In Agra soil,

Anant Rao *et al.*<sup>172</sup> mentioned 30 lb. N per acre as optimum for wheat, the performance of ammonium sulphate being closely followed by castor cake.

A high stimulating effect of sodium (as NaCl) was reported by Idnani<sup>173</sup> as a supplementary dose when wheat was fertilized with ammonium sulphate.

Chela<sup>174, 175</sup> reported that in sandy loam soils in the Punjab, ammonium sulphate at 40 and 100 lb. N per acre gave better yields of wheat and maize respectively than FYM. Ammonium phosphate, was however, somewhat better than ammonium sulphate. Raheja *et al.*<sup>176</sup> obtained an yield response of 7.4-8.1 lb of maize grain per lb. of N applied within 20-60 lb. N dose per acre in Delhi soil.

In fairly fertile Karnal soil, nursery manuring with 1000-2000 md. of FYM per acre produced stout and vigorous seedlings but the ultimate yield of paddy was not improved unless manured with ammonium sulphate (Gupta and Relwani<sup>177</sup>). Chavan *et al.*<sup>178</sup> in Bombay obtained higher responses in paddy yield by increasing the ammonium sulphate dose from 32 to 64 and 96 lb. N per acre.

According to Bhatta and Narayanan,<sup>179</sup> nitrogen requirement of sugarcane in Mysore is fairly high, varying from 300-450 lb. per acre. Nitrogen supplied wholly in organic form was found to be inferior to ammonium sulphate. A dose higher than 500 lb. N decreased the sugar content. Rao and Narasimhan<sup>180</sup> observed that in south, ammonium sulphate should be applied to cane in 1½ to 4½ months for best results.

Neelakantan and Raja<sup>181</sup> reported that ammonium sulphate applied broadcast or by drilling to local cotton in Kovilpatti was better than oilcakes, the most profitable dose being 20 lb. N per acre. Similar observations were made by Bederker *et al.*<sup>182</sup> Das<sup>183</sup> found ammonium sulphate giving the best yield of jute out of all the forms of nitrogen tried. Pal *et al.*<sup>184</sup> observed that an increase in nitrogen dose from 40 to 60 lb. per acre resulted in a small rise of nitrogen in the fibre with a decrease in quality. For cigar tobacco, the optimum nitrogen dose was 100-150 lb. per acre as ammonium sulphate (Khemchandani *et al.*<sup>185</sup>).

Gokhale<sup>186</sup> and Gokhale and Bhattacharyya<sup>187</sup> examined the effect on soil acidity and calcium content due to ammonium sulphate manuring to tea in upper Assam soil. The fertilizer applied at the rate of 90 lb. N per acre for 13 years lowered the soil pH to 4.15 from 4.55 and the change was noticeable down upto 3 ft. In another experiment for 20 years the pH came down to 4.00. The calcium depletion proceeded at the rate of 22.34 lb. CaCO<sub>3</sub> per acre for every 100 lb. of ammonium sulphate from 3 ft. depth of the profile. Gokhale's experiments<sup>188</sup> showed indirectly that tea in acid soils when manured with ammonium sulphate absorbs nitrogen in NH<sub>4</sub> form and the tea plant also takes much of its nitrogen as free nitric acid.

Zende and Kalbhor<sup>189</sup> studied the mobility of nitrate ions in the soil applied at 4 in. depth as fertilizers. The mobility is dependent on the solubility of different fertilizers and their rate of nitrification and also upon the type of negative ion and its organic or inorganic nature.

Ammonium nitrate applied to paddy at 20, 40 and 60 lb. N per acre by Digar and Gupta<sup>190</sup> significantly increased the grain and straw yields, its use for 4 years having no effect on soil composition.

Rao *et al.*<sup>191</sup> found Chilean nitrate to compare favourably as fertilizer for paddy in South Kanara soils having a pH of 5.53. In Malabar (soil pH 4.85), Mahadevan and Gopala-krishnan<sup>192</sup> found it to be somewhat inferior to ammonium sulphate, recording only 88 per cent of the yield due to the latter fertilizer. Pillai and Purushothaman<sup>193</sup> reported that ammonium sulphate recorded slightly higher yields of *ragi* (*Eleusine coracana*) than Chilean nitrate, though the difference was slight and not significant.

According to Khanna *et al.*,<sup>194</sup> the average increases in sugarcane yield in the state of Bihar due to the application of ammonium chloride and urea at 20, 40 and 80 lb. N worked out to 2.8, 5.4 and 8.3 and 3.4, 6.8 and 10.7 tons per acre respectively and with ammonium sulphate the respective yield increases were 3.4, 6.5 and 10.7 tons.

Govindarajan and Rao<sup>195</sup> designed simple and inexpensive

equipments to inject liquid ammonia under pressure into the soil. The ammonia so injected is largely retained in the soil, the loss by diffusion ranging between 1-2 per cent and it undergoes rapid nitrification in a week to 10 days. Field trials for 3 seasons showed liquid ammonia to be as effective as ammonium sulphate on same nitrogen level for paddy and sugarcane.

### Phosphatic fertilizers

In lowland Gangetic alluvium (pH 6.7), bonemeal applied to paddy for 10 years did not increase the yield in the first 3 years but later on both grain and straw yields increased significantly, as reported by Digar and Mandal.<sup>196</sup> Nitrogen and phosphorus in the crop and soil and organic carbon and exchangeable Ca in the soil increased appreciably. Raheja and Bains<sup>197</sup> observed that in seven phosphate deficient areas, the direct response of paddy to 1½ and 3 md. of superphosphate were 3.96 and 4.90 md. per acre respectively and residual effects were 2.16 and 3.47 md. In another trial, 3 md. of superphosphate gave 2.93 md. of extra maize per acre and residual effect of 1.51 md.

Phosphate application showed an acceleration in growth and maturity and also increase in the yield and oil content of mustard (*Brassica juncea*) in trials by Sen and Sarkar.<sup>198</sup>

Sen and Bains<sup>199</sup> and Singh *et al.*<sup>200</sup> reported that phosphate manuring of *berseem* for 3 years gave high yields of the legume fodder and the effect of building up of soil fertility was observed from the increase in the wheat yield for the following 3 years. Superphosphate alone or with FYM was better than FYM alone and a dose of 64 lb. of P<sub>2</sub>O<sub>5</sub> per acre to *berseem* was found to be most remunerative. Khan<sup>201</sup> pointed out that the increase in the yield of *berseem* by increasing the P<sub>2</sub>O<sub>5</sub> dose from 60 to 120 lb. per acre was not significant and the yield was largely influenced by irrigation frequencies. According to Singh,<sup>202</sup> phosphate manuring increased the *berseem* yield, nodulation, weight of nodules and induced more nitrogen in tops and roots. It increased the nitrogen in crop and also in the soil.

Superphosphate increased the yield of lucerne fodder by 44.86 per cent and 50 lb. P<sub>2</sub>O<sub>5</sub> dose per acre was found to be most

economical by Jadeja and Patel.<sup>203</sup> Kanwar and Grewal<sup>204</sup> reported that phosphate in acidic, calcareous and alkaline soils of the Punjab readily changed to slowly soluble forms, maximum change occurring within one day. Equilibrium between the readily and slowly available fractions got established in 28 days. Bonemeal, rock and tricalcium phosphates gradually changed to more soluble form in acid soils and less soluble form in calcareous and alkaline soils.

### Potassic fertilizers

There was no advantage in potassic fertilization of paddy grown in Hyderabad soils where adequate nitrogen and phosphate were applied, according to Desai *et al.*<sup>205</sup> According to John,<sup>206</sup> potash manuring in Bombay and the Punjab showed significant response in the yields of orange, wheat and maize in the second and third years of cropping. High yielding strains responded better to potash than low yielding ones.

From the results of trials conducted in cultivators' fields, Raheja *et al.*<sup>207</sup> found potash fertilization to be profitable for paddy in Bihar, Andhra, Madras, Kerala and Mysore and for wheat in Bihar, Punjab, Delhi and Rajasthan. Maize yields were increased in the Punjab, response being much more in the hybrid than in local corn. Sugarcane and potato profitted from potash application in Bihar and Punjab and coconut and tapioca in S. India Application of 40 lb. K<sub>2</sub>O per acre to pruned bushes of tea reduced very materially their mortality.

### Compound fertilizers.

In the saline coastal soil, bonemeal applied with ammonium sulphate gave significant increase in paddy yield but not when used alone, reported by Banerjee and Digar<sup>208</sup>. Bhaumik<sup>209</sup> reported that in wastelands in the DVC area, ammonium sulphate increased the yield of paddy, which increased further with phosphate. In a machine reclaimed area, both N and P singly were ineffective but very high yields were obtained by their combined addition. Pawar and Murthy<sup>210</sup> found that in heavy soils of Telangana, phosphate is essential for paddy along with nitrogen. On an alluvial clay soil, Digar<sup>211</sup> obtained the maximum yield of paddy for 10 years

with a mixture of 100 md. of FYM and 30 lb. of N as ammonium sulphate. Digar and Gupta<sup>212</sup> working on the lower Gangetic alluvium clay soil for 4 years reported that paddy yield was significantly increased with FYM or town compost and bonemeal. For short duration paddy in black cotton soil (clay loam), Desai *et al.*<sup>213</sup> recommended a combined application of phosphate with nitrogen in the ratio 1 : 0.75.

For irrigated wheat in Saurashtra, Motwani<sup>214</sup> recommended a dose of 20 lb. N and P<sub>2</sub>O<sub>5</sub> per acre as optimum. A dose of 30 lb. N and 20 lb. P<sub>2</sub>O<sub>5</sub> per acre was found to be most efficient for wheat in light loam alluvial soils of U.P. by Singh.<sup>215</sup>

Choudhuri and Choudhuri<sup>216</sup> obtained the highest yield of potato by applying 80 lb. N and K<sub>2</sub>O plus 160 lb. P<sub>2</sub>O<sub>5</sub> per acre with 30 by 8 in. spacing. Fertilizing, however, had no effect on tuber size. Bose<sup>217</sup> studied the effect of N, P, and K on growth, yield and oil content of mustard. Phosphate with nitrogen showed a significant effect on flowering, seed formation and oil content. In the manuring of tapioca in Kerala, Chadha<sup>218</sup> observed significant interaction between the doses of nitrogen and potash. NPK at 40-40-80 lb. per acre gave the best results. The yield of *Colocasia esculenta* (*Arvi*) in loamy soil of Jullundar was appreciably increased by NPK manuring, the most profitable combination found by Purewal and Dargan<sup>219</sup> being 100 : 50 : 50 per acre.

Manuring of sugarcane with compost, ammonium sulphate or a mixture of the two, all in combination with superphosphate was found by Farooque and Shrivastava<sup>220</sup> to have similar effects on gur quality. Thakur and Chatterjee<sup>221</sup> investigated that under normal soil conditions in Bihar, ammonium sulphate with superphosphate had a greater stimulating effect on the germination of sugarcane as compared to compost. When the soil was very sandy, compost with phosphate was the best, ammonium sulphate, on the other hand, having a very depressing effect. According to Muthuswami,<sup>222</sup> a mixture of groundnut cake and ammonium sulphate was significantly better than ammonium sulphate alone for sugarcane.

Malik<sup>223</sup> reported a 90 per cent increase in the yield of the forage crop, *Heteropogon contortus*, grown at 4000 ft. with a 15:5

NP mixture. Both ammonium sulphate and superphosphate were found to be beneficial by Singh and Sahasrabuddhe<sup>224</sup> for groundnut and *arhar* under rainfed conditions.

### Fertilizer application

According to Abichandani and Patnaik,<sup>225</sup> large nitrogen losses particularly from surface applied fertilizers were observed due to denitrification in the reducing zone of water-logged soils, whereas sub-surface application of ammonium sulphate and nitrate was superior in all respects. The recovery of NH<sub>4</sub>-N with sub-surface placed ammonium sulphate was 88 per cent compared to 40 in surface applied fertilizer. Menon<sup>226</sup> observed that placement of ammonium sulphate as pellets with ten parts of soil in paddy fields in Kerala reduced the leaching loss and increased the efficiency of ammonium sulphate from 20-30 to 50-70 per cent. Raheja and Chowdhury<sup>227</sup> obtained an additional 7.2 md. of paddy per acre by placement of N and P fertilizers over broadcast application.

Drilling of NP fertilizer at 2½ in. depth increased the yield of maize by about 2 md. per acre over surface broadcast application in a trial by Raheja *et al.*<sup>228</sup> Khan<sup>229</sup> working with maize also found that placement of NPK fertilizers through plough sole method was better than broadcast and placing in open furrows. As regards foliar spray of NPK fertilizers to potato, Mariakulandai *et al.*<sup>230</sup> did not find foliar feeding as adequate without soil supplement of plant food.

Paddy seeds soaked in 10—20 per cent solution of K<sub>3</sub>PO<sub>4</sub> for 16 hours (3 parts of seed to 1 part of solution) till all the solution was absorbed, induced a significantly consistent increase in grain yield, as high as 23 per cent over no manure, in a number of experiments by Narayanan *et al.*<sup>231</sup> K<sub>2</sub>HPO<sub>4</sub> gave a little increase in yield whereas KH<sub>2</sub>PO<sub>4</sub> was not effective.

### Soil amendments ; Lime

Sen and Taneja<sup>232</sup> observed that addition of 1.5 tons of gypsum did not affect the moisture retention capacity in soil. It helped in stopping nitrogen loss in soil receiving large application of legume litter as manure. Sen and Rewari<sup>233</sup> found that the salinity of a sandy loam soil increased due to a portion of gypsum

being dissolved in soil water extract. Increase in soluble Mg was noted due to displacement of exchangeable Mg by Ca. Large increase in soluble Na was observed but no increase in soluble K.

Profitable returns from the application of lime to paddy in the acidic soils of Coorg were reported by Subramaniam and Vardarajan<sup>234</sup> but only in 71—100 inches rainfall zone and not in the lower or higher rainfall zones.

### **Soil phosphorus tests for soil testing work**

Datta and Kamath<sup>235</sup> have reported comparative performance for several of the more common rapid soil tests for phosphorus on a wide variety of Indian soils. The moisture equivalent of these soils varied from 6.9—47.7 per cent, pH from 5.0 to 8.8 and  $\text{CaCO}_3$  from nil to 6.5 per cent. However, highly acid soils were not used and most of the soils were slightly acid, neutral or alkaline. In a comparatively smaller number of cases samples from field experiments were used. Percentage yield response and 'A' values were used for comparing the various method. In general, correlation of the soil test values with yield response was much better for green-house studies than for the field. The performance of Olsen's  $\text{NaHCO}_3$  method was the best and most satisfactory. In almost all cases highly significant correlations were obtained with this method. The method appeared equally applicable for soils growing paddy. The prediction value on the logarithmic scale for both wheat and paddy was as high as 80 per cent. The limits for low, medium and high values for both wheat and paddy were 20, 21—50, 50 lb.  $\text{P}_2\text{O}_5$  per acre respectively.

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## GENERAL MICROBIOLOGY

### Including Antibiotics

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The review covers the period from December 1957 to December 1958. Considerable additions have been made to our knowledge of fundamental and applied microbiology. Biochemistry and metabolism of microorganisms including the effect of antibiotics, have engaged the attention of many of the workers. Considerable work has also been done on the documentation and control of plant diseases. The largest number of communications has been on morphology and taxonomy and the fewest on newer techniques. Three new antibiotics have been reported, two being antifungal and one, a member of the actinomycin group, showing antitumour activity.

In August 1958 the Hindustan Antibiotics at Pimpri started a quarterly house journal generally devoted to all aspects of the antibiotics industry and in the first two numbers appearing this year, stimulating review articles on general microbiology with special reference to microbiological aspects of penicillin production and screening of soils<sup>52,166</sup> have been published.

Papers presented at the Antibiotics symposium held at Pimpri in March 1956 have been published in book form by the Council of Scientific and Industrial Research, New Delhi. These papers have already been reviewed in an earlier (1956) volume of this series.

During the year five important and comprehensive reviews have appeared, three by Indian and two by foreign authors in Indian journals. Das Gupta<sup>1</sup> has dealt with the history of botanical researches in India, Burma and Ceylon with special reference to mycology and plant pathology, in a well documented monograph. Raghavendra Rao<sup>5</sup> has given an excellent review of the acetic acid bacteria. Couch<sup>4</sup> and Brown<sup>3</sup> have dealt with soil microbiology and ecological significance of microorganisms, while Kalyanasundaram and Braun<sup>2</sup> have reviewed Indian work of 10 years on cotton wilt.

## Morphology and Taxonomy

There have been a number of new records<sup>15,19,21,23,26,44</sup> of known species of fungi. Systematic studies of fungi of various regions have been carried out by several schools of workers. Thus, Thind *et al.* have continued their studies on the Pezzizaceae, Clavariaceae, Thelephoraceae, and Myxomycetes of the Mussoorie hills.<sup>18,19,22,28,36</sup> Agnihothrudu<sup>35</sup> has reported on the fungi of North-east India with special reference to the tea estates of Assam. Joshi<sup>44</sup> has continued work on the fungi of Ajmer, and Salam *et al.*<sup>19</sup> on the fungi of Hyderabad. *Septobasidium theae* has been recorded from Assam<sup>33</sup> and a new sp. of *Discosia* from Darjeeling.<sup>48</sup> A beginning has been made on the study of the Indian *Aspergilli*, a systematic treatment of which group is long overdue.<sup>38</sup> New host records of several rusts have been reported.<sup>26</sup> New species of *Cercospora*,<sup>51</sup> *Piricularia*<sup>12</sup> and *Pyrenophaeta* parasitic on *Dolichos biflorus*<sup>47</sup> and *Parodiella*<sup>34</sup> have been notified. An undescribed Myxomycete has been recorded from Assam by Agnihothrudu.<sup>35</sup> He also has proposed *Thozetellopsis* as a new genus of the Tubulariaceae from Assam.<sup>17</sup>

Chiddarwar<sup>37</sup> has listed additions to our knowledge of the fungal flora of Bombay including three spp. of *Parodiella*, one of *Metarrhizium* and three of *Oidium*. Subramanyam<sup>7,29</sup> has continued taxonomical studies on Hyphomycetes and lists several new species including *Arthrobotryum nilgirensis*, *Ceratosporella deviata*, *Periconia nilgirica*, *Stachybotrys nilgirica* and *Drumophoma giris*. He has indicated the relationship of *Drumophoma* to *Melanographium*. Bakshi *et al.*<sup>15</sup> have reported *Polyporus leucospongiae* and a new record for *Trametes* on Deodar timber.

On comparative morphology and cytology there have been relatively few papers. Payak<sup>20,25</sup> has given an excellent cytological account of the life cycle and spore forms of *Uromyces hobsoni* Vize noting short cycling tendencies and inheritance of endo condition. He has also described the nucleolar chromosomes in *Scopella gentilis*. Joshi and Reddi<sup>49</sup> have dealt with the comparative taxonomy of species of *Uromyces* on *Indigofera*

species. Singh<sup>9</sup> has made comparative studies of *Fusarium moniliforme* Sheldon, and *Cephalosporium sacchari* Butler.

In studies of living yeast nucleus Thyagarajan<sup>46</sup> has noted the reaction of vacuole and nucleus to neutral red and Royan<sup>53</sup> has reported a nuclear membrane with chromocentres and an extra vacuolar nucleus as evidenced by haematoxylin staining.

Mohanty and Das<sup>43</sup> have recorded leaf spot of safflower as due to *Ramularia carthami* and Mohanty and Behera<sup>39</sup> have described sesame blight caused by *Alternaria sesame* (Kawamura) Comb. Nov. Bakshi and Balwant Singh<sup>8</sup> have recorded the occurrence of *Merulius himantoides* in India. Jagtap<sup>14</sup> has made an interesting study of the entomogenous fungus *Metarrhizium anisopliae*.

Studies on the rhizosphere on pigeon-pea and sugarcane have been continued by Agnihothrudu and Srinivasan in Assam and Madras respectively.<sup>32,30,42</sup> They have recorded a sp. of *Chaetoceratostoma* and a new genus *Hyalostachybotrys* gen. nov.<sup>30</sup> Srinivasan and Vijayalakshmi<sup>6</sup> have recorded *Piricularia oryzae* on *Arundo donax* L.

Subba Rao and Joshi<sup>27</sup> have listed and identified micro-organisms isolated from spoiled preserves and Sarkar<sup>53a</sup> the bacterial flora of toned milk. Chona *et al.*<sup>21</sup> have listed several new records and species of fungi and Thirumalachar<sup>52</sup> has reviewed mycological aspects of penicillin production.

### Physiology and Ecology

Rangaswamy<sup>54</sup> has discussed the multifarious role of microorganisms in soil including their relative effect on each other and the changing condition of the soil and the effect of both on agriculture.

Srivastava and Gupte<sup>77</sup> have surveyed the human skin flora with special reference to fungi and have reported on the effect of diaryl sulphones, sulphoxides and sulphides on *Trichophyton rubrum*. They have also<sup>68</sup> made a comparative physiological study of four strains of yeast isolated from human excreta and the inhibitory *in vitro* effect of Nystatin on them. Sharma and Shukla<sup>62</sup> have attempted to differentiate primary and secondary

colonies of yeast by  $E_h$  time curves. This is a novel physical method of differentiation of the primary from the secondary colonies, which may find considerable use in strain selection and deficiency mutant studies, and drug resistance.

Banerjee and Sarkar<sup>60</sup> continuing their work on heterothallism find three principal types of reactions between monosporic cultures, compatible, antagonistic and intermediate and that these reactions are independent of the presence or absence of clamp connections.

Continuing his work on oxygen consumption of *Penicillium chrysogenum* Thom., Ghosh<sup>66</sup> has reported that penicillin yields are unaffected by only 0.3 vol. of air/vol. of broth in large fermentors. Grewal<sup>65</sup> has studied the effect of various carbon compounds on different aspects of growth and morphology, observing among other things that the number of setae in the acervuli of *Colletotrichum papayae* are affected by the type of carbon compound in the medium. A great deal of systematic study in this direction would help the taxonomist in deciding on morphological criteria for species delineation.

Iyengar and Ganju<sup>64</sup> have irradiated spores of *Phycomyces blakesleanus*, with  $P^{32}$  at various levels and have noted changes varying from temporary dwarfing at lower levels (20  $\mu$ c.ml.) to pronounced biological and genetic effects including sterility and branching of sporangiospore at higher levels (70  $\mu$ c. ml.). Branching is of interest as the genus is identified with an unbranched sporangiospore. Bedi<sup>74,75,76</sup> has discussed the role of stale products and the effect of presence of other organisms in sclerotial formation in *Sclerotinia aclerotiorum* (leb.) de Bary. He has used ultra-violet light to speed up the germination of sclerotia and apothecial formation.

Sulphur requirements of *Phyllosticta* sp. have been studied by Tandon and Bilgrami<sup>56</sup> and Agarwal<sup>61</sup> has reviewed the literature on sulphur and phosphorus requirements of fungi. Tandon and Srivastava<sup>59</sup> and Tandon<sup>55</sup> have described the effect of hormones and vitamins on the growth and morphology of *Alternaria tenuis*, *Pestalotia malorum* and *P. psidii*. Saraswati Devi<sup>58</sup> has discussed the essentiality of trace element to soil fungi

and Iswaran<sup>57</sup> on the non-availability of inorganic nutrients in agar media.

Nambudripad *et al.*<sup>67</sup> have reported on the utilization of riboflavin, biotin and nicotinic acid by *Lactobacilli*. Banerjee<sup>69</sup> has discussed the nucleic acid content of *E. coli*., in relation to dissociation. Narsinga Rao and Natarajan<sup>72</sup> have made a comparative physiological study of strains of *Pseudomonas aeruginosa*. Sreenivasan<sup>70</sup> has reported that 50% of marine bacteria is capable of reducing nitrates to nitrites and nitrogen and that *Pseudomonas* was foremost in nitrate reduction.

### Biochemistry and Metabolism

Sadasivan<sup>110</sup> in his presidential address to the Indian Botanical Society discussed the implications of recent advances in our knowledge of moulds, their metabolites and interaction of these with host tissues, as vivo-toxins, as constituents of the rhizosphere, and in altering soil conditions, thus affecting plant physiology and pathology. He has given an attractive picture of the expanding horizons of mycology. Venkateswaran<sup>79</sup> has reviewed generally the fermentation industries and Saksena<sup>83</sup> writes on the recent advances in penicillin production.

Ramakrishnan *et al.*<sup>106</sup> have reported a new growth factor for *Mycobacterium tuberculosis* from coconut milk which speed up the growth of the organism *in vitro* and seem to protect the infected animal when given *in vivo*. Suryanarayanan<sup>86</sup> has recorded absolute heterotrophy to vitamins by *Pircularia* sp. and *Sclerotium oryzae* and has found no correlation between vitamin deficiencies and virulence.

On biosynthetic studies of fungi and bacteria, Irani and Ganapathi<sup>82</sup> have recorded the stimulatory effect of glycerol in penicillin synthesis by the mould *P. chrysogenum*. Deshpande and Ganapathi<sup>81</sup> have made extensive studies on the biosynthesis of penicillin and reported the effect of various carbon sources. They have employed with great facility the resting cells of the mould. Jagjit Singh and Iswar Dutt<sup>80</sup> have found that fat synthesis by *Aspergillus nidulans* reaches the peak with 0.3% ammonium nitrate in the medium. Raina and Ramakrishnan<sup>85</sup>

have listed malic and oxalic acids as intermediate acids during citric acid biosynthesis by *A. niger* in M.Y.G. medium. Radhakrishnamurthy and Sarma<sup>109</sup> have studied the optimum conditions for the biosynthesis of riboflavin by *Eremothecium ashbyii*. 2-3, Butanediol formation by bacteria has been studied in *Serratia marcescens* by Bahadur and Dube<sup>84</sup> and in *Aerobacter aerogenes* by Ranganayaki and Bahadur.<sup>102</sup> The latter investigation indicates that minerals,  $\text{CaCO}_3$ , phosphates and neutral pH favour the formation of 2-3 butanediol. Mujumdar and Bose<sup>101</sup> have studied the utilization of a variety of amino-acids on the growth and antibiotic production by *Bacillus subtilis*. Nitrogen fixing properties of *Azotobacter* have been studied by Sen and Iswaran<sup>103,63</sup> who find that heat inactivates the fixing capacity while freezing increases it.

Freitas and Bhat<sup>92</sup> have recorded the cellulose decomposing capacities of *Nocardia* and Pruthi and Srivastava<sup>78</sup> the microbial spoilage of passion fruit during storage. Some of the fungi listed are, *Penicillium expansum*, *Fusarium oxysporum*, *Aspergillus niger* and *Rhizopus nigricans*.

Das<sup>100</sup> has studied some biophysical characters of *Pasturella septica*, and Srikantan *et al.*<sup>95</sup> have studied the aldolase activity of virulent and avirulent strains of *P. pestis*. They have also studied the dehydrogenase activity in relation to pathogenicity.<sup>96</sup> Continuing the work on enzyme make up of *Vibrio cholerae* Sagar *et al.*<sup>98</sup> have reported ribonuclease activity. Mohanrangam and Manjrekar<sup>97</sup> have shown that antibiotics penicillin and chloramphenicol have profound staphylophosphatase and staphylocoagulase activities which are correlated with the pathogenicity of *Staphylococcus*. As to mould enzymes, Gupta<sup>94</sup> has studied the pectic enzymes in parasitic fungi and has recorded them in *Pythium de Baryanum*, the damping-off fungus.

In a series of papers, Natarajan<sup>87,89,107,108</sup> has dealt with the C/N metabolism of soil fungi including effects of cyanide, nitrite, sulphur and the influence of carbon sources on nitrogen metabolism, mechanism of nitrate utilization and transaminase activity in *Fusarium vasinfectum*. Reduction of nitrates by *Fusaria* is unaffected by carbon sources or potassium cyanide.

Rao<sup>93</sup> studying the glucose metabolism of *Streptomyces griseus* finds that arsenate inhibits growth and streptomycin formation and iodoacetate inhibits these as well as glucose oxidation, while fluoride stimulates streptomycin formation and DNP the oxidative activity. Tandon and Bilgrami<sup>90</sup> has studied the utilization of oligosaccharides by *Pestalotia* species by chromatographic techniques. They use three nitrogen sources in combination with different oligosacharides. Sanwal<sup>99</sup> has studied the transaminase systems of *Fusarium*.

Ghatak *et al.*<sup>104,105</sup> have continued their studies of the enzyme make up of *Salmonella typhosa* in respect of aldolase and catalase activity in relation to virulence. Chatterjee and Ghosh<sup>91</sup> working with cell free extracts of *Leishmania donovani* have found trans-glucosidase and transfructosidase which enable successful use of sucrose as a carbon source.

### Plant Pathology

Chona<sup>121</sup> has listed some of the diseases of sugar-cane reported in India, and while noting that none of them are of general epidemic nature he recommends study and planning of breeding and control measures for a possible eventuality. Govinda Rao<sup>119</sup> has reviewed the progress of plant pathology in Andhra Pradesh. Bagchee<sup>132</sup> has reviewed five years work on causes and control of forest diseases in India. Bhargawa and Gupta<sup>142</sup> have initiated work on market diseases of vegetables and fruits and have reported on *Pythium* infection of beans.

Ghosh and Mukerjee<sup>131</sup> have reported the tip rot of *Hibiscus cannabinus* due to *Trichosphaeria* sp., and Mathur and Mathur<sup>134</sup> on fruit rot of *Citrulus vulgaris* by *Fusarium oxysporum* establishing its pathogenecity. Varadarajan<sup>120</sup> has reported a blight and die back, caused by *Alternaria tenuis* of *Rauwolfia serpentina*.

Sharma and Asthana<sup>145</sup> have reported on *Pythium aphenodematum* causing fruit rot of *Memordia dioica*. Damodaran and Ramakrishnan<sup>148</sup> have found *Gloeosporium* sp. as a banana anthracnose causal organism in a multiplicity of strains. Sukhapure *et al.*<sup>140</sup> have worked out the pathology of *Fusarium*

wilt of garden peas, Pawar and Patel<sup>144,156</sup> the *Phomopsis* blight of brinjal, *Alternaria* leaf spot of castor, and Rangaswamy and Venkata Rao<sup>141</sup> the *Alternaria* blight of cluster beans. Kulkarni *et al.*<sup>113</sup> have studied the pathology of *Macrophomina* stem break disease of cotton, and Ghosh<sup>139</sup> has established the causal organism of jute anthracnose as *Colletrichum corychorum*.

Kale and Prasad<sup>138</sup> have recorded a new variety of *Phytophthora parasitica* var. *sesami* based on host specificity of the organism. Hegde and Bhide<sup>133</sup> have noticed for the first time watermelon wilt caused by *Fusarium oxysporum* f. *niveum*. Sharma and Merh<sup>128</sup> have recorded for the first time *Piricularia* disease on *Echinochloa*. Das<sup>146</sup> has noted a *Cercospora* leaf spot of *Aegle marmelos* and Srinivasan<sup>127</sup> *Pythium* root rot and its influence on chlorosis complex of sugar-cane. Tandon and Bilgrami<sup>125</sup> have reported leaf spot disease caused by *Phyllosticta artocarpina* on *Artocarpus heterophyllus*. Chopra<sup>116</sup> reviews the red rot disease of sugar-cane and Kar and Singh<sup>115</sup> report a new leaf disease. Gill<sup>112</sup> reports on the pathology of a new linseed powdery mildew from Punjab.

Bhaktavatsalu and Balasubramanyam<sup>124</sup> have discussed problems and methods of forecasting early blight of potato in Nanjanad. Baruah *et al.*<sup>114</sup> working on the susceptibility of potato to *Fusarium* have found low iron content conferring high resistance to the host. Mehr and Kulkarni<sup>111</sup> have reported on the study of varietal resistance of linseed to wilt and rust. Sreekanthiah and Joshi<sup>135</sup> have recorded *Trichothecium* sp. as a hyper-parasite on *Puccinia graminis tritici* (Pers) Eriks and Henn.

On host parasite relationship studies Chattopadhyay and Chakrabarti<sup>137</sup> have correlated the degree of resistance to *Helminthosporium oryzae* by paddy, to  $\text{SiO}_2$  in leaf sample, thickness of cuticle, epidermal cell and percentage of bulbiform cells per unit leaf area. Laxminarayanan,<sup>148,129</sup> continuing studies on the physiological aspects of *Fusarium* wilt of cotton, has recorded the formation of pectin methyl esterase by the parasite and derangement in the distribution of amino acids in the host due to infection.

Patel and Patel<sup>117</sup> have reported a new *Xanthomonas* wilt

of *Cyamopsis tetragonoloba*, Rajagopalan and Rangaswamy<sup>130</sup> on the bacterial leaf spot of *Pennisetum typhoides* and Ravindranath *et al.*<sup>123</sup> on a bacterial wilt from Hyderabad.

### Applied Microbiology and Miscellaneous

Gupta<sup>153</sup> has surveyed the human intestinal bacteriophage and discusses the dynamics of plaque formation. Work on fungicides and use of antibiotics as fungicides have attracted some attention. Kaleswara Rao and Govinda Rao<sup>153</sup> have reported successful control of the powdery mildew of cluster beans with Bordeaux mixture, Cerasan and Cupravit. Kandaswamy<sup>154</sup> has reported the control of turmeric leaf spot disease; Govinda Rao and Subbiah<sup>149</sup> have found merculine at 1 oz. in 5 gal. effective against *Sclerotium rolfsii*.

Rangaswamy and Damodaran<sup>152</sup> have reported *in vitro* effectiveness of mycostatin against three fungus parasites of banana. They also report that the antibiotic is fungistatic to *Deightoniella torulosum* at lower and fungicidal at higher concentrations, time of exposure being an important factor. Velankar<sup>151</sup> has reported inhibition of marine bacteria by aureomycin. Joshi<sup>164</sup> has discussed the genetics of resistance to diseases and pests.

Aswathnarayana and Subramanyam<sup>167</sup> have evaluated the standards used in the study of yeast nucleus. Dutta and Bose<sup>155</sup> have typed the serological reactions of sensitive and resistant strains of *Micrococcus pyogenes* var. *aureus* to penicillin.

Hazra *et al.*<sup>168</sup> have described a rapid method for estimating cellulolytic activity of fungi using chlor-zinc iodide as a colour reagent. Royan<sup>160</sup> has used an excellent pretreatment (fixation) with iodine-formol-acetic acid solution for staining yeast nucleus. Rangaswamy<sup>158</sup> has described an agar block technique for isolating pythiaecious organisms from soil and Bose<sup>161</sup> a method for isolating from culture media. Srinivasan<sup>159</sup> has described a technique for isolating pathogenic *Pythium* cultures from soil using host tissue baits. Gopalakrishnan<sup>166</sup> has reviewed the methods of screening of soils for antibiotics. Tsuda<sup>162</sup> has described fine structure of cytoplasmic granules of *Penicillium chrysogenum* cells with the aid of electron microscope.

Vasudeva *et al.*<sup>170</sup> have reported a new extra-cellular antibiotic, bulbiformin from culture filtrates of *Bacillus subtilis*; magnesium was found necessary for production of the antibiotic. Ahmad *et al.*<sup>168</sup> have reported a new actinomycin from a *Streptomyces* isolate, and Thirumalachar and Ghosh<sup>165</sup> have made a preliminary report on an actinomycin produced by a *Streptomyces* sp.

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## FISH AND FISH PRODUCTS

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This review is restricted to the work on chemistry and technology of fish and fish products, reported from different laboratories in India during the year 1958.

During the year some progress has been noted in the use of antibiotics in the preservation of fish. Working with sea-fish kept in ice containing varying amounts of aureomycin, Velankar and Kamasastri<sup>1</sup> observed the beneficial effect of aureomycin over that of ordinary ice after the first week of storage. A lower bacterial and trimethylamine content of fish muscle was observed in those kept in 5 p.p.m. aureomycin ice. The number of psychrophiles was also much lower. Velankar<sup>2</sup> studied the effect of aureomycin on the growth of 48 bacterial species isolated from the marine environment and from sea-fish. He found that Gram negative achromatic rods, *Vibrio*, *Flavobacterium*, *Bacterium* and *Bacillus* were sensitive to as low as 2 p.p.m. *Pseudomonas* and other species producing yellow, red and violet pigment were sensitive only at higher levels of 5 to 20 p.p.m. The cocci were more resistant than Gram-negative rods. The bacterial counts of fish slime on aureomycin containing agar were reduced to 1/3 at 2 p.p.m. and to 1/25th at 5 p.p.m. level. In the case of sea-water, the count was reduced to 1/5th at 2 p.p.m. and 1/20th at 5 p.p.m. levels. At higher levels, the count was zero. The results indicated that use of aureomycin in ice or in 'initial dips' might be beneficial to the storage life of wet fish.

Moorjani *et al.*<sup>3</sup> in a preliminary study on mackerel, *Rastrelliger kanagurta*, subjected to frozen storage, reported that development of rancidity to be the principal factor leading to deterioration of stored mackerel. In their experiments the fish was eviscerated, washed, sealed in MSAT cellophane paper and stored at 4°F (-20°C). Total nitrogen and non-protein nitrogen and peroxide values of lipids in fish muscle were determined together with organoleptic evaluation. The important change that took place in the fish muscle during the experimental period was the develop-

ment of objectionable off-flavour suggestive of fat spoilage. There was not much loss of tenderness or development of toughness in the fish muscle even after five months of storage. The off-flavour appeared after three months and was quite pronounced at the end of five months. There was concomitant development of peroxides with a decrease in soluble protein nitrogen. It was suggested that the denaturation of lipo-protein might have caused liberation of lipids thereby rendering them more susceptible to oxidation.

Suryanarayana *et al.*<sup>4</sup> carried out a study on the method of pit curing of fish to determine the optimum conditions for the cure and the keeping quality of the cured products and also to evaluate the different chemical tests for the detection of spoilage during storage. Mackerel was chosen for the investigation which also included observations on the more common method of wet salting. The authors noted that there was a large increase in the free aminoacid content of the fish during the curing, correspondent with softening of the tissue. The total volatile basic nitrogen also increased during the curing period and reached more than 500 mg. per cent. The optimum period for the cure was found to be two days in the case of gutted mackerel salted in the ratio 1 : 5.

Moorjani *et al.*<sup>5</sup> have assessed the quality gradings of fresh-water fish by determining the changes in the volatile reducing substances and the total volatile base content. The varieties used by them were "Gende" (*Barbus carnatiensis*), "Kooralu" (*Barbus dubious*), "Arja" (*Cinchina fulungee*), "Bale" (*Wallago alter*) and "Machallu" (*Labeo* Spp.). The value for total volatile base remained relatively constant during the prime quality, increased to some extent during the second quality phase, and increased significantly during spoilage. They consider that these values could be used as an index of fish spoilage provided the initial value for the fish in question was known and that maximum increase permissible for fresh-water fish of the species investigated would be about 10 mg.% volatile basic nitrogen. The volatile reducing substances (VRS) expressed as micro equivalents reduction per 5 ml. juice increased coincident with the onset of spoilage indicated by organoleptic tests and by bacterial counts. The values increased more rapidly with progressive storage. Fish fillets were reasonably fresh upto a value of 8-10 for VRS, while

slight off-odours were detected for VRS values below 15. A value greater than 20 indicated definite off-odours.

Studies on the composition of the fish tissue protein and the distribution of the non-protein nitrogen in fish tissue have been the subject of a few papers published during the year from Indian laboratories. Velankar and Govindan<sup>6</sup> have investigated the distribution of non-protein nitrogen in a few marine fishes, crustaceans and molluscs. The non-protein nitrogen constituted about 10 per cent of the total nitrogen in the teleosts, 20 per cent in the crustaceans and molluscs and over 30 per cent in the elasmobranches. Higher levels of free  $\alpha$ -amino nitrogen, of the order of 300 mg. N/100 gm. of wet muscle, were found in the crustaceans and molluscs as compared to 20 to 40 mg. N/100 gm. of wet muscle in the normal fish. The amount of trimethylamine varied widely in different samples of fresh fish. Bose *et al.*<sup>7</sup> determined quantitatively, by paper chromatographic method, the amino acid composition of flesh from Bombay Duck (*Harpadon nehereus*) Ribbon (*Trichiurus Spp.*) and Shark (*Carcharinus gangeticus*). It was observed that the muscle tissues of the above mentioned three varieties of fish contain all the essential amino acids except in the flesh of Bombay Duck in which methionine is absent. Joshi *et al.*<sup>8</sup> determined the amino acid composition of edible muscle of ten representative animals including fish, by the paper chromatographic and microbiological assay methods. They observed that the general pattern of distribution of amino acids in the muscles of mammals, aves, reptiles, amphibia and fish was almost similar.

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## HUMAN NUTRITION

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Subrahmanyam *et al.*<sup>1,2</sup> carried out a feeding experiment for a period of six months on young girls to study the effect of replacing rice in a poor vegetarian diet by tapioca macaroni made up of 60 parts of tapioca flour, 15 parts of low-fat groundnut flour and 25 parts of wheat semolina. There was no significant difference in the increase in height, weight and red-cell count between the control and the experimental groups. The nutritional status of seven children in the tapioca-macaroni group improved as compared with three children in the rice group.

The metabolism of nitrogen, calcium and phosphorus was studied in the same subjects during the course of the above experiment.<sup>3,4</sup> The usual conventions were followed for the selection of the subjects from the two groups. All the children were in positive nitrogen, calcium and phosphorus balance. The mean daily retentions of nitrogen, calcium and phosphorus were higher in the case of the tapioca macaroni diet group, but the difference between the two groups was not statistically significant.

Subrahmanyam *et al.*<sup>5</sup> studied the effect of partial replacement of rice in a poor vegetarian diet by enriched wheat macaroni in young children on the general health and nutritional status. The statistical analysis of the results showed a significant improvement in the weight and haemoglobin level of children receiving the enriched wheat macaroni over those in the control group. Thirteen children in the experimental group showed improvement in nutritional status as against only four in the control group.

Joseph *et al.*<sup>6</sup> studied the effect of feeding poor rice diets based on husked, undermilled and milled rice on the metabolism of nitrogen, calcium and phosphorus in children. All the subjects maintained positive nitrogen balance. The calcium retention on husked rice diets was very much lower than that on the other diets

probably due to the high phytic acid content in husked rice. The data clearly indicated that consumption of poor rice diets containing husked rice was likely to lead to negative calcium balance. All the subjects maintained positive phosphorus balance. Phosphorus retention on husked and undermilled rice diets was higher than that on milled rice diet.

Joseph *et al.*<sup>7</sup> studied the effect of poor rice diet based on ragi on the metabolism of nitrogen, calcium and phosphorus in children. The data indicated that all the subjects maintained positive nitrogen, calcium and phosphorus balance.

Jayalakshmi and Gopalan<sup>8</sup> compared the incidence of tuberculin positivity in a group of mal-nourished children on the one hand and apparently normal children of the general population on the other. It was observed that in the age group under six years there was no significant difference with regard to the incidence of tuberculin positivity as between the mal-nourished and control subjects. In the age group between 6 and 10 years, the incidence of tuberculin positivity was actually greater in the control group than in the mal-nourished group. The incidence of tuberculosis and Kwashiorkor among children of the same socio-economic group was also compared. It was found that the peak incidence of tuberculosis preceded that of Kwashiorkor, but the period between 1 and 3 years in which Kwashiorkor was the major problem was also the period of maximal incidence of childhood tuberculosis.

Narasinga Rao and Gopalan<sup>9</sup> studied nitrogen, calcium and phosphorus metabolism in experimentally induced tuberculosis in guinea pigs and in tuberculosis on human subjects. They found that guinea pigs on high protein diet recovered from the negative balance phase following on infection more rapidly than those on the low protein diet. All the eight human cases of tuberculosis showed large positive nitrogen, calcium and phosphorus balance. Body weight changes sometimes led to misleading indication of the status of nitrogen retention. Positive nitrogen balance was found to be associated with good prognosis. Using the criterion that the protein intake resulting in maximal nitrogen retention was the optimal protein requirement, cases of tuberculosis required not

less than 100 g. of protein per day.

In a follow-up study of fifteen cases of Kwashiorkor, Srikantia *et al.*<sup>10</sup> found that the dietary pattern of the children investigated was nearly the same as at the time of their first admission with symptoms of Kwashiorkor. Despite the lack of improvement of the diet, only two of fifteen children suffered from relapses. This observation would support the view that for the actual development of Kwashiorkor, dietary inadequacy of protein might not be the sole determinant. The superimposition of acute infective episodes would probably be necessary to tip the children into the florid state of Kwashiorkor. In the absence of such episodes the children might be able to maintain themselves in a state of mild or marginal mal-nutrition as evidenced by the growth failure, moon-face and the hypo-albuminaemia in several cases. The growth failure observed in these cases might actually be a means of adaptation of the organism to the protein inadequacy. A suggestive evidence in favour of this view was that while the body weights of some of the children investigated here were definitely lower than even the averages obtaining for the apparently normal children of the same socio-economic group, and while the absolute daily intake of protein was considerably less than the accepted normal allowances of children of comparable age, the protein intake considered in relation to the body weight approached the recommended figure. The freedom from fresh attacks of frank Kwashiorkor in a majority of cases investigated could be attributable to a relative diminution in protein requirement and relatively greater immunity to infection in the later years of childhood.

None of the cases revealed evidence of hepatic fibrosis. Protein deficiency type of vacuolation of the liver cells which could be corrected by dietary protein supplementation was the predominant finding. Liver function, as judged by the serum cholesterol and serum albumin levels appeared to be unimpaired.

Padmavati *et al.*<sup>11</sup> investigated the dietary fat, serum cholesterol levels with incidence of atherosclerosis and hypertension. The incidence of hypertension was found to be 1.2% in industrial workers in Delhi and 2.6% in rural population, that of coronary artery disease was nil as judged from the history and about 1.3% in

Najafgarh if the small number of electro-cardiograms taken are considered.

There was no correlation between the serum cholesterol levels and the dietary fat or cholesterol intake. The serum cholesterol level tended to increase with age but the rise was very gradual. Gopalan<sup>12</sup> investigated the effects of protein supplementation and of so-called galactagogues on lactation in women of poor socio-economic group. In women subsisting on 60 g. of protein daily and having an output of breast milk of the order of 13-20 oz. per day, protein supplementation had no beneficial effect as far as lactation was concerned. None of the so-called galactagogues like garlic, cotton seed, tamarind or leptadin was found to have a significant effect on lactation.

Basak<sup>13</sup> determined the urinary elimination of total nitrogen, sulphur and phosphorus of four human adults of different ages and body weights. Significant positive correlation was found between nitrogen and sulphur, and nitrogen and phosphorus in endogenous urine. The average value of nitrogen, sulphur and nitrogen: phosphorus ratios was calculated as  $7.5 \pm 0.20$  and  $7.2 \pm 0.41$  respectively. The difference between the N:S ratio of endogenous urine and of the fasting urine (and tissue proteins) and the reconciliation of the former with the N:S ratio of non-protein tissue constituents showed that endogenous nitrogen metabolism did not represent the disintegration of tissue proteins, but it was associated with and originated from the catabolism of non-protein nitrogenous tissue constituents.

The endogenous nitrogen metabolism seemed to be interrelated to the endogenous metabolism of sulphur and such that the phosphorus cellular catabolism of 7 atoms of nitrogen of non-protein tissue constituents was concomitant to the cellular catabolism of 1 atom of sulphur and 1 of phosphorus from the same source.

Narasinga Rao *et al.*<sup>14</sup> carried out nitrogen balance studies on six women belonging to poor socio-economic group during lactation at three levels of protein intake. They found that the subjects were in the neighbourhood of nitrogen balance on an intake of 61 g. protein per day. Increasing the daily protein intake

to 99 g. induced considerable positive nitrogen balance in all the subjects. Increasing the intake further to 114 g. protein per day had little additional effect.

Gopalan and Ramanathan<sup>15</sup> investigated the effect of feeding large quantities of different types of fat on serum cholesterol concentration in a group of human volunteers and monkeys. While butter and hydrogenated vegetable fat caused a sustained increase of serum cholesterol concentration, sesame oil had no such effect. Diets deficient in proteins and / or calories also reduced the serum cholesterol concentration.

Pasricha<sup>16</sup> carried out a survey of dietary intake in a group of poor pregnant and lactating women consisting of 100 pregnant and 70 lactating women and found that their dietaries were inadequate in calories, proteins and calcium. Further, there was no direct correlation between the intake of the mother and the birth weight of the infant in the group investigated. There was a direct relationship between the intake of the nutrients and the per capita income of the family per consumption unit. The proportion of animal protein in the protein intake was less than one in five.

Padmavati *et al.*<sup>17</sup> carried out a diet survey in Delhi by the 'questionnaire' and 'weightment of foods' methods and concluded that the two methods were comparable for most purposes. Dietary pattern in 1957 among the rural poor were better than among the urban poor though both were considerably short of recommended dietary allowances. Among the high socio-economic groups the diet was still inadequate in respect of animal proteins, although milk consumption was adequate. A comparison of the 1957 data with previous ones showed a drop in the consumption of cereals and pulses, both in urban and rural groups but no increase in the intake of protective foods.

Mukherjee and Sarkar<sup>18</sup> estimated nitrogen content, catalase activity, iron content, cholinesterase and alkaline phosphatase activities of liver obtained at biopsy material of patients suffering from nutritional oedema and of babies with Kwashiorkor. In all cases liver nitrogen was low on admission to the hospital and increased with recovery. The catalase activity amounted to 3.6 ml. O<sub>2</sub> per mg. nitrogen per second on admission and 1.6

ml. O<sub>2</sub> per mg. nitrogen per second after recovery. The iron content decreased from 46 mg per 100 g. on admission to 17 mg. per 100 g after recovery, showing a definite correlation with the catalase activity. Cholinesterase was low on admission by 70% compared to the value obtaining after recovery. Alkaline phosphatase activity was 200% higher on admission than after recovery. This might be a reflection of the attempt on the part of the organism to adjust the metabolism to the protein deficiency.

Rao and Rao <sup>19-21</sup> carried out a comprehensive nutrition survey of the rural population in Pennathur. Based on the clinical and biochemical data obtained in the survey, the most common deficiencies related to nutrition have been discussed. The deficiency states associated with lack of vitamin A, riboflavin and ascorbic acid were found most frequently. Anemia of the hypochronic, microcytic type was common. This might be due to a low intake of iron, protein or other nutrients, an interference with the absorption and utilization of iron or a high prevalence of intestinal parasitism. From the qualitative and quantitative aspect of the survey, it was apparent that the Pennathur diet was essentially a cereal diet, the chief cereals being cholam, rice, ragi and cambu. Milk, meat and fish being almost absent from the diet, the diet was essentially a poor vegetarian diet. The diet of a large number of the families was deficient in calories and protein. The protein intake was 50-75% of the recommended allowance. The percentage of families with an intake of less than 50% of the recommended allowance of riboflavin, vitamin A, ascorbic acid, fats, calcium and iron was very high. The intake of thiamine and niacin was also below recommended levels. Phosphorus intake was adequate. The primary reason for the dietary inadequacy seems to be the low income. The authors suggested a nutritional improvement programme by health education in proper food habits, methods of working and supplementing green leafy vegetables.

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## BOOK REVIEWS

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**Recommendation of the International Commission on Radiological Protection (adopted September 9, 1958).**

(Pergamon Press London) 1959, Pp. vi + 18. Price 3/6 d.

The International Commission on Radiological Protection (ICRP) established in 1928 has been periodically publishing its recommendations, although under different organisational forms, and the present slim volume formulates its latest ideas on the subject of radiation protection. As newer and better knowledge accumulates in the future, in regard to sources of radiation hazards and the effects of ionising radiation, the current recommendations are certain to undergo appropriate changes. However, the present report has the distinction that it is based on more rational ground than before, although it would appear to be restrictive in operation. At any rate, it would greatly help to allay some of the fears and doubts of the chronic pessimist in regard to exposure hazards.

Hitherto, the so called maximum permissible dose had been expressed in terms of a short period of time (0.3 rem/week). This has now been reduced to 3 rems/13 consecutive weeks and the definition of permissible dose altered to include the two different types of possible long-term somatic effects, viz., leukemia and shortening of life span, as also the genetic effects. In view of this, the maximum dose has been prescribed for individuals and for population groups, occupationally exposed or otherwise. In either case, limits have been set on the basis of dose accumulated over a period of years rather than in terms of a weekly dose that would be received over an indefinite period of time. Further, separate recommendations have been made in regard to dose for different organs or tissues which have been for this purpose categorised into three groups depending upon their radiosensitivity. The Commission has also recommended a limit of 5 rems for the genetic dose from all man-made sources of radiation and activities, except medical procedures. Details in regard to the rationale followed

are provided in separate sections following a prefatory review, viz., basic concepts and maximum permissible dose. The volume ends with an appropriate section dealing with the general principles regarding working conditions which will be found useful by persons in charge of controlled areas.

The Pergamon Press is to be congratulated not only for the excellent get-up of the publication but also for undertaking the financial responsibility for its distribution at modest price and without copyright restriction.

R. K. MALLER.

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### THE METHOD OF ISOTOPIC TRACERS APPLIED TO THE STUDY OF ACTIVE ION TRANSPORT

### LA MÉTHODE DES INDICATEURS NUCLÉAIRES DANS L'ÉTUDE DES TRANSPORTS • ACTIFS D'IONS

1<sup>er</sup> Colloque De Biologie De Saclay, 1958 (Pergamon Press, London) 1959, Pp. ii + 196, Price 60 s. net.

During the last 10 years the study of active ion transport using radioactive materials across animal membranes, nerve and muscle have made good progress; it is gratifying to note that Pergamon Press have taken interest in publishing the available data presented at the Congress of Saclay, in July 1958 in the form of a book.

Dr. E. J. Conway has presented the unified redox-pump theory and this concept has been utilised to explain the mechanism of gastric secretion and sodium excretion in yeast. Dr. I. M. Glynn in another article has brought out the common feature dominating the permeability of nerve, muscle and red blood cells in line with the redox-pump theory and has shown how this concept can explain the mechanism of action of cardiac glycosides. Dr. D. Nachmansohn has discussed the role of acetylcholine in understanding some aspects of nerve conduction. The possibility of alteration of molecular structure of the receptor proteins has

been postulated to explain the activity of analogues of acetyl choline. Dr. A. K. Solomon has studied the ion water transport in the kidney of the amphibian *Necturus* and has shown that sodium is actively transported out of the proximal tubule. Dr. H. H. Ussing in his paper on the active ion transport across epithelial membranes concludes that in the epithelial cells of the frog-skin the mechanism of the sodium-potassium pump is active and argues that transitions always exist between active and passive processes.

In this symposium several of the world's best known authorities on the subject have taken part and it is difficult to single out any of the papers as more important, as most of them furnish information of some importance on this most elusive subject. On the whole, the book is extremely informative and stimulates further research and so deserves a place in a research laboratory.

A. S. RAMASWAMY.

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## **PATHOGENESIS AND IMMUNOLOGY OF TUMOURS**

*Edited by*

**G. V. VYGODCHIKOV**

Translated from Russian

**R. CRAWFORD**

(Pergamon Press, London) 1959, Pp. 258. Price 80 s.

This volume dedicated to L. A. Zil'ber in honour of his sixtieth birthday, presents a collection of articles by leading Russian specialists. "Current problems in the pathogenesis of malignant tumours and their immunology" is the main theme discussed under four broad classifications: Pathogenesis of malignant tumours; Antigens; Immunity, antibodies and vaccination and Electron microscope investigations.

The new concept to regard cancer and all other true tumours as proliferative reactions on the part of the organism to various toxic factors, extrinsic or intrinsic and not as one fundamentally differing from other diseases in its reactivity, advanced by Petrov merits the attention of all oncologists. Experimental evidence for the virus etiology of tumours, the distinction between the

immunity to tumour cells and immunity to tumorigenic virus, heterotransplantation and metastasis of Brown-pearce rabbit carcinoma and the virus nature of the milk factor are important contributions in the field of carcinogenesis.

The difference in the antigenic composition of papilloma and its terminal stage of cancerous tumour, the nature of specific antigens in the leucosis of man, existence of relationship between leucosis and sarcoma in fowls, attempts to obtain tumour viruses for prophylactic use, demonstration of specific antigens in cancerous tissues of man and other species of animals and the techniques for fractionation of specific antigens, described by various authors are definite advances in cancer research.

Demonstration of antibodies to various types of tumours, the role of spleen in the formation of antibodies and attempts at artificial immunization against experimental cancer, present new approaches for the control of cancer.

M. SIRSI.

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## QUANTITATIVE METHODS IN HUMAN PHARMACOLOGY AND THERAPEUTICS

Proceedings of a symposium held in London, 1958, sponsored by Biological Council's co-ordinating Committee for symposia on Drug Action series

*Edited by*

D. R. LAURENCE, M.D.

(Pergamon Press, London) 1959. Pp. 253. Price 45 s.

The advances in the science of therapeutics have been phenomenal during the last two decades. Chemotherapy by antibiotics and synthetic antibacterials, anticoagulants in intravascular thrombosis, vitamin B<sub>12</sub> in anaemia, tranquillizers in anxiety neurosis and mental affections, and the adrenal cortico steroids in collagen diseases are but a few examples of the successful outcomes of pharmaceutical research.

Unlike the experimentation on animals where many variables could be controlled, the drug trials in clinical practice are beset with innumerable modifying influences. Practical problems

associated with the clinical trials for assessment of drug actions are many. Rigorous application of the basic principles used for statistical evaluation in pharmacological experiments has to be adhered for proper appraisal of efficacy and comparative evaluation.

"Quantitative methods in human pharmacology and therapeutics" is a timely publication specially at the present juncture, when innumerable new products claiming to be more effective and less toxic than the older remedies are being introduced at an inconceivably fast rate. The improved methods in assessing the effects of treatment, by carefully planned therapeutic trials has been chiefly responsible notably in establishing the therapeutic merits of some drugs beyond any doubt but has also shown the need for such experimentation, before any claims could be made. This aspect of the study with selected examples has been dealt with by various authors in 'physiological measurements in man', measurement of the effects of thyroid hormones, of neuromuscular blocking agents, of drugs on gastro-intestinal tract and of analgesics. These studies emphasize that there is no escape from the necessity of scientific pharmacological investigations on man directly, in the normal state, in states of distress, in disorders and diseases.

The extremely difficult field of analysis of drug action on the higher centres of the nervous system is attempted in the chapters on the measurement of some muscular concomitants of emotion, effects of drugs on performance, incentives, and motor activity and on measurements of subjective states.

The series of articles on the newer statistical methods applicable to human pharmacology and therapeutics present the desirable incursions of statistics into the medical and pharmacological domain. Human experimentation demands, more than do most research activities, the effective team work of different specialists. The contributions of statistical design in successful completion of clinical trials and the methodology adopted have been described with appropriate examples. Groupings, use of randomized and incomplete randomized blocks, latin squares, sequential analysis and the statistical analysis of semi-quantitative results give a glimpse of the potentialities in the application of statistics to clinical trials under varying conditions.

The preoccupation with the laudable desire for statistical irreproachability should not blind one to the virtues of small scale trials. Each trial could, with sufficient care and ingenuity, be made to yield quantitative results of surprising precision. In fact future advances in trials of potential new drugs largely depend on these 'preclinical testings'. These aspects are very well discussed in the 'limitations on the design and conduct of small scale drug trials', and it is heartening to note that even when experimental design is not beyond reproach, drug trials, even on small groups of subjects, could be made to yield results of great value.

The ethics of experimentation on human beings is extremely controversial. Considering the species variations in drug action, even after all the pharmacological studies on animals, the final evaluation depends on human trials. The ten Nuremberg rules shown in the appendix offer a general guidance for such experiments on human beings. This delicate subject has been critically discussed in the chapter on "Ethics of clinical trials". Does the end justify the means adopted? What kind of consent is required to alter the sick person's status for the benefit of Research? Is some form professional sanction or moral censorship essential to control undesirable trials? Could the trials so far carried out by investigators in various countries give specific leads for future methodology? "The ethics of human trials" presents a stimulating approach to the entire problem of human experimentation.

M. SIRSI.

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## AN OUTLINE OF DEVELOPMENT PHYSIOLOGY

By CHR. P. RAVEN.

Second Revised Edition. (Pergamon Press, London) 1959,  
224 pp. Price 25 s.

This is a delightful introduction to the fascinating problem, as to how a fertilized egg consisting of a nucleus and cytoplasm develops into a co-ordinated and integrated system of organs and tissues of an organism. The extensive multiplicity of the adult is traced to the intensive multiplicity appearing in the egg following

fertilization. "In the phenomena of life all order is a consequence of previous order..... We must accept this order as an essential characteristic of life, a basic phenomenon, of which no causal explanation is possible."

The presentation is simple and lucid and should appeal to those anxious to learn the fundamentals of this subject.

M. K. SUBRAMANIAM.

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## **FOURTH INTERNATIONAL CONGRESS OF BIOCHEMISTRY**

**Vol. XIV, Transactions of the Plenary Sessions**

Editor: W. AUERSWALD

(Pergamon Press, London, 1959) 299 pp. Price 75 s.

This volume presents the proceedings of the Plenary Sessions of the IVth International Congress of Biochemistry held in Vienna in September 1958. Summaries of the various symposia held during the Congress Sessions are also included. The topics discussed run a whole gamut from the Biochemistry of the Central Nervous System and Viruses to Biochemistry of Antibiotics and Steroids, Morphogenesis and Insect Biochemistry.

In his address to the Plenary Sessions on the 'Chemistry of Heredity', Chargaff gives an account of the various ways in which the phenomena of heredity may be subjected to biochemical research. He emphasizes the need for a detailed study of the order of alignment of the nucleotide monomers in a nucleic acid chain in order to decipher the code-scripts used in the transmission of biological information.

Braunstein's address on 'Some aspects of Chemical Integration of Nitrogen Metabolism' summarizes the present status and outlook of problems under investigation in his laboratory for the last two decades. He traces in a masterly fashion the inter-relations between nitrogen metabolism and the energy supplying processes of the cell. The functions of the dicarboxylic amino acids and amides in the assimilation and dissimilation of nitrogen

and the role of pyridoxal catalysis in the integration of Nitrogen Metabolism are described.

This is followed by summaries of symposia which succeed remarkably well in retaining the spirit and content of the deliberations held. The titles of the summaries are given below:

1. Carbohydrate Chemistry of substances of Biological interest by Wolfram.
2. Biochemistry of Wood (in German) by Kratzel.
3. Biochemistry of the Central Nervous System (in German) by Brücke.
4. Biochemistry of Steroids by Erich Mosettig.
5. Biochemistry of Antibiotics by Chain.
6. Biochemistry of Morphogenesis by Nikerson.
7. Biochemistry of Viruses (in German) by Broda.
8. Biochemistry of Proteins by Neurath.
9. Physical Chemistry of Biologically important High Polymers (in German) by Kratky.
10. Blood clotting factors (in German) by Deutsch.
11. Metabolism of Vitamins by Umbreit.
12. Insect Biochemistry by Levenbook.

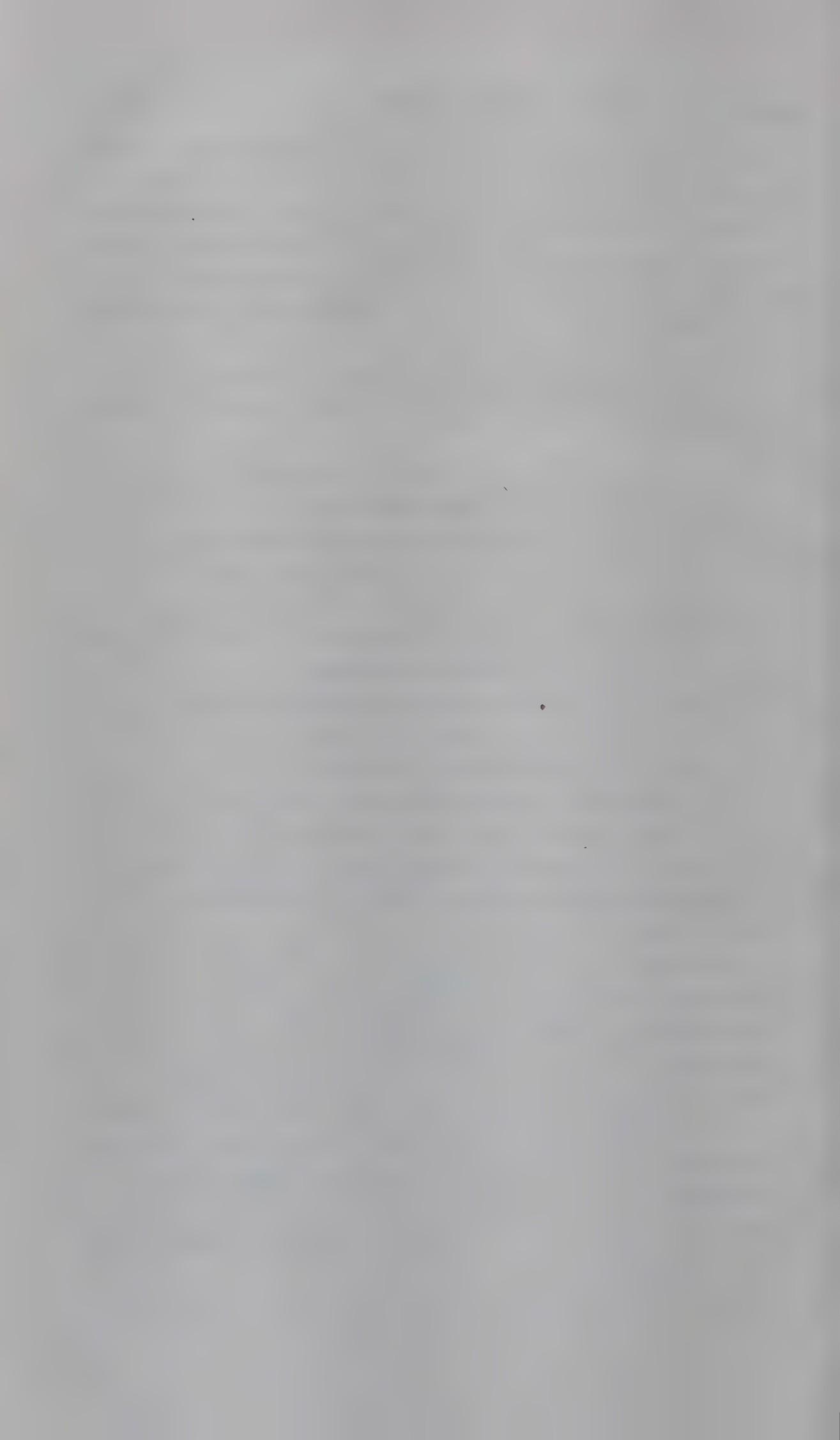
In summarizing the symposium on Proteins, Neurath critically examines the progress made in the last several years in the elucidation of structure, biological activity and biogenesis of proteins and anticipates trends which further progress is likely to follow.

Levenbook in his article on the symposium on Insect Biochemistry gives a brief sketch of the rapid strides made in recent years in this area of research. The mechanism of the complicated process of metamorphosis, host specificity of insects and the isolation of insect hormones are dealt with in detail.

A bewildering body of useful information is packed in this volume and the reviewer has no doubt that it would be an invaluable reference book to all research workers in Biochemistry.

C. S. VAIDYANATHAN.

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